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STUDIES ON THE LABORATORY POPULATIONS OF *DROSOPHILA VIRILIS*

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INTRODUCTION

D. virilis and *D. americana* are closely related species of the *virilis* group. Both are found in America, but they are ecologically isolated, the former being a domestic form, while the latter is a wild form (Patterson, 1942). Their hybrids have never been found in natural populations, though hybridization in laboratory is possible.

There is a very strong reproductive isolation between the two species, as shown by the low hatching rate of the hybrid eggs and the low viability and partial sterility of the hybrids (Patterson and Stone, 1949) The fertility of hybrid males is influenced by the mode of combination of chromosomes of different species When such a male has a Y-chromosome of *D. americana*, he must have a certain autosome complex of the same species for fertility (Patterson, Stone and Griffen, 1940, 1942); the hybrid male having chromosome sets of *D. virilis* except for Y and a fourth chromosome of *D. americana*, is also sterile, when he is backcrossed to *D. virilis* (Chino, 1943). The same kind of X–Y relation for fertility and viability has been confirmed for hybrid males having the Y-chromosome of *D. virilis* (Oshima, 1948, 1949).

However, the fertility of hybrid females is normal or slightly low regardless of their chromosome combinations (Stone, 1947). When the hybrid females having the chromosome set of *D. virilis* except for one X-chromosome of *D. americana*, are placed with *D. virilis* males in a population cage, the hybrids among her offspring gradually decrease in number, and are eventually eliminated from the population. Analysis of factors responsible for such a change has clarified to some extent the mechanism of reproductive isolation.

Comparing the results of the first and second experiments to be described in this paper, one may find that in the laboratory population selection favoring isolation between the two species is unexpectedly strong.

THE HYBRIDS BETWEEN *D. americana* AND *D. virilis* IN THE POPULATION CAGE

The chromosome complex of the hybrid female between *D. americana* and *D. virilis* may gradually be replaced by the chromosome complex of *D. virilis* by

repeated backcrossing to *D. virilis* males. *D. virilis* used in this mating, is the mutant strain involving *yellow* and *apricot* genes in the X-chromosome. In salivary gland chromosomes of the hybrid, there is a complex overlapping inversion extending from the middle of the X-chromosome to the proximal inert region. As crossing-over is almost entirely suppressed in this inverted region, an *apricot* gene included in this region is only rarely transferred to the X-chromosome of *D. americana*. On the other hand, the distal part of the X-chromosome of *D. virilis* in which the gene for *yellow* is located, freely pairs with the corresponding part of the X-chromosome of *D. americana*, and recombination may take place in this part. Thus, a hybrid strain having the X-chromosome, of which the distal part is formed by *D. virilis* chromosome and the proximal part by *D. americana* chromosome and the autosomal set of *D. virilis*, was obtained. This strain can be maintained by mating the females having yellow body with the males having yellow body and apricot eyes. These females and males were placed in a population cage of the type used by Wright and Dobzhansky (1946) in their experiment. The culture medium was renewed about once a month. All the existing flies were etherized and classified according to their phenotypes, and living flies were returned to the cage.

TABLE 1. *Non-disjunction of the hybrid females* ♀ $X^{v}X^{v-a}$(Y) (*y*) × ♂ Oyachi strain (*D. virilis*)

F_1 ♀♀ (+)	Except. (*y*)	♂♂ (*y*)	(*y-ap*)	Except. (+)
361	79	133	170	75
Rate of non-disjunction	17.95			19.84
♂ $X^{v-a}Y$ (*y*)/♂ $X^{v}Y$ (*y-ap*)			0.78	

Non-disjunction of hybrid females and viability of hybrid males

The hybrid yellow females of this strain, mated with males of the strain from Oyachi, Hokkaido, produced a large number of non-disjunctional progeny, as shown in table 1. The rate of this non-disjunction reached about twenty times the standard rate of the secondary non-disjunction in *D. virilis*. This suggests that the majority of the hybrid females had the sex-chromosomes of the XXY type. This supposition is sustained by the fact that these exceptional males have normal fertility. The number of

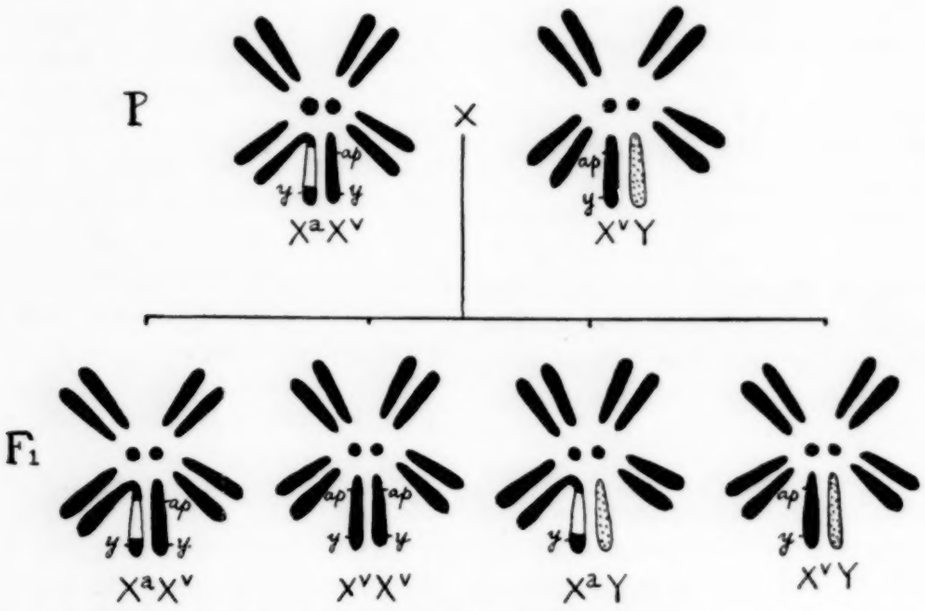

FIG. 1. Karyotypes of the flies (P) placed in the population cage at the start of experiment and the F_1 generation.

TABLE 2. *The experiment of "female multiple choice"*

	Female	Male	Period of mating	F_1 ♀♀ (*y-ap*)	(*y*)	♂♂ (*y-ap*)
Hybrid strain	♀ (*y-ap*)	♀ X^vX^{v-a}(*y*) × ♂(+) F_1♂(*y*) F_1♂(*y-ap*)	1st week	104	0	101
			2nd week	153	0	118
			3rd week	71	20	88
			Total	328	20	307

	Female	Male	Period of mating	F_1 ♀♀ (*y-ap*)	(*y*)	(+)	♂♂ (*y-ap*)
Pure strain	♀ (*y-ap*)	♀ (*y-ap*) × ♂(+) F_1♂(+) F_1♂(*y*) F_1♂(*y-ap*)	1st week	2	14	120	106
			2nd week	0	32	96	95
			3rd week	1	33	82	82
			4th week	8	22	74	84
			Total	11	101	372	367

y males among the offspring of these hybrid females was 0.78 times that of the corresponding *y-ap* males, and the former hatched two or three days later than the latter. This difference in number between the two kinds of males will be discussed in the later section.

Multiple choice by females

The males having different kinds of X-chromosomes among the offspring of the hybrid females can be distinguished by the markers they contain, as indicated in the previous section. These *y* and *y-ap* males were placed with virgin *y-ap* females of *D. virilis* in a small vial, and the females were left to choose their mates. The males that had mated with the females can be told by the phenotype of the F_1 females: if all the F_1 females have apricot eyes, the *y-ap* males were chosen as the mates, while if all of the F_1 females have red eyes, the father was

TABLE 3. *Number of living and dead flies in the population cage*

Date	Count	Living flies	Dead flies	Number of survivors	Number of substitution	Rate of substitution
Jun. 7	1	215				
Jul. 3	2	802	—	(215)	(587)	0.73
Jul. 28	3	952	221	581	372	0.39
Aug. 20	4	1211	363	590	621	0.51
Sep. 12	5	1239	463	748	491	0.39
Oct. 3	6	907	442	797	110	0.12
Oct. 25	7	757	355	552	205	0.27
Nov. 18	8	1004	236	521	483	0.48
Dec. 12	9	1188	321	683	505	0.42
Jan. 10	10	1031	482	706	325	0.31
Feb. 8	11	815	239	792	23	0.02
Mar. 9	12	617	302	513	104	0.16
Mar. 24	13	463	257	360	103	0.22
Mean		862			327	0.34

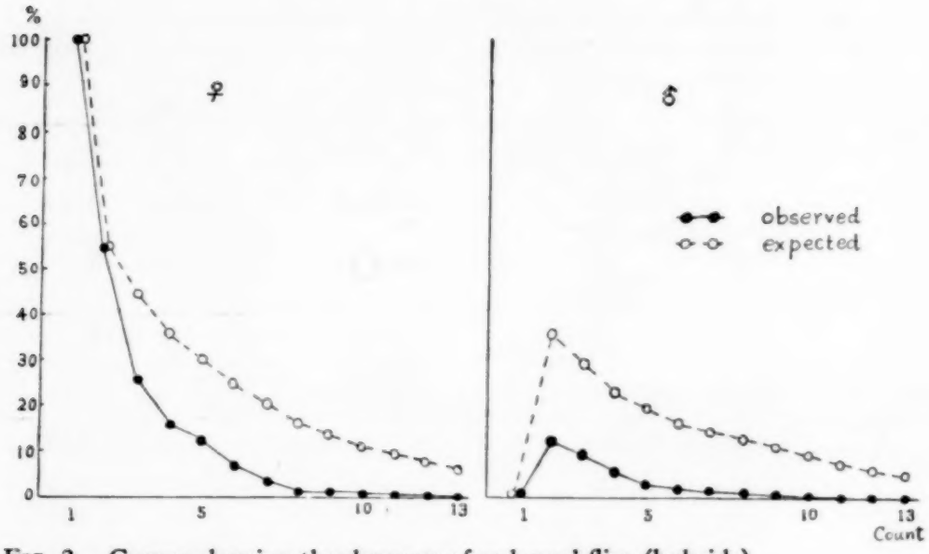

FIG. 2. Curves showing the decrease of red-eyed flies (hybrids).

y. Three males of each phenotype and seven *y-ap* females were placed in a small vial, and, after a week, the females were transferred to a new vial, and the phenotype of their offspring was examined. Controls were set up using males of *D. virilis* strain. The results are shown in table 2.

Competition leading to extinction

This experiment was begun on July 7, 1949, with 108 hybrid females of yellow body-color and 107 *D. virilis* males of yellow body-color and apricot eyes, and terminated on March 24, 1950. The cage was kept in a constant temperature room held at 25° C. The flies in the cage were examined as stated in the previous section, and the results shown in table 3 were obtained. The individuals substituted between the counts were about 34 per cent of the total number of the population. The flies were divided according to their sexes and eye colors; their respective numbers are shown in table 4. As shown in this table, the hybrids with red eyes have been eliminated from the population. The hybrid males in particular are unexpectedly few in every count, and have been completely eliminated by the 10th count.

The percentage of heterosynapsis (B) in the meiosis of the hybrid females may be calculated from the incidence of non-disjunction (E) (table 1) by using Bridges' formula: $B = \frac{100+E}{400\ E}$ (Bridges, 1916). Thus, the percentage of heterosynapsis amounts to 61 per cent and that of homosynapsis about 39 per cent. Accordingly, the proportion of gametes produced from the hybrid females should be as follows:

	From Homosynapsis (XX-Y)		From Heterosynapsis (XY-X)			
Gametes	XY	X	X	XY	XX	Y
Proportion	19.50	19.50	15.25	15.25	15.25	15.25

Thus, the incidence of the gametes having X or XY is 19.50 + 15.25 = 34.75 per cent and that of the exceptional gametes having XX or Y is 15.25 per cent.

The ratio of the eggs with the gene for red eye-color (ap^+) to the eggs having the *apricot* gene (*ap*) in the above experiment (table 1) is 0.78:1.00. On the other hand, it is shown by the result of the experiments of multiple choice by

TABLE 4. *Number of red-eyed and apricot-eyed flies*

Count	♀ red-eyed flies	♀ apricot-eyes flies	♂ red-eyed flies	♂ apricot-eyed flies
1	108	0	0	107
2	241	195	46	320
3	136	375	40	402
4	110	549	32	520
5	88	592	18	541
6	27	385	12	483
7	12	361	7	377
8	6	488	6	504
9	8	633	2	545
10	3	585	1	442
11	1	471	0	343
12	1	366	0	250
13	0	257	0	206

females, that all sperm had either the *ap*-gene or the Y-chromosome. Accordingly, the expected values of each genotype of both sexes in the next generation will be as below:

Thus, the expected percentage of red-eyed and yellow-body flies are as follows:

$$\text{Expected } ♀ \ (y): \frac{29}{54} \times 100 = 53.7 \ (\%)$$

$$\text{Expected } ♂ \ (y): \frac{16}{46} \times 100 = 34.8 \ (\%)$$

The ratio of the ap^+, ap, ap^+/ap and Y eggs in the next generation may be calculated as follows:

ap^+ eggs: $0.29 \times 2 \times 0.35 \times 0.78 = 0.16$

ap eggs: $0.29 \times 2 \times 0.35 + 0.25 \times 2 = 0.70$

ap^+/ap eggs: $0.29 \times 2 \times 0.15 = 0.09$

Y eggs: $0.29 \times 2 \times 0.15 = 0.09$

As the relative number of the sperms having ap and Y in the next generation is expected to be the same, the expected values (percentage) of each phenotype of both sexes should be as follows:

		Normal eggs		Exceptional eggs	
		$\overset{ap^+}{(0.35 \times 0.78) = 0.27}$	$\overset{ap}{0.35}$	$\overset{ap^+/ap}{0.15}$	$\overset{Y}{0.15}$
Sperms	1.00 ap	0.27 ap^+/ap	0.35 ap/ap	—	0.15 ap/Y
	1.00 Y	0.27 ap^+/Y	0.35 ap/Y	0.15 ap^+/ap/Y	—

The number of females in the population was always larger than that of males, the mean sex-ratio being about 54 ♀ : 46 ♂. The frequency of each phenotype of the next count may be calculated by assigning 54 to females and 46 to males according to the proportion given above:

♀ ♀ *yellow:* $54 \times \dfrac{0.27 + 0.15}{0.27 + 0.15 + 0.35} = 29\%$

♀ ♀ *yellow-apricot:* $54 - 29 = 25\%$

♂ ♂ *yellow:* $46 \times \dfrac{0.27}{0.27 + 0.35 + 0.15} = 16\%$

♂ ♂ *yellow-apricot:* $46 - 16 = 30\%$

♀ (y-ap): $54 \times \dfrac{0.70}{0.16 + 0.70 + 0.09} = 39.79 \ (\%)$

♀ (y): $54 \times \dfrac{0.25}{0.95} = 14.21$

♂ (y-ap): $46 \times \dfrac{0.79}{0.95} = 38.25$

♂ (y): $46 \times \dfrac{0.16}{0.95} = 7.75$

As to the number of living flies in this count, it is probable that 66 per cent of the flies in the previous generation have been added to the 34 per cent in the new generation (the mean rate of substitution).

The ultimate expected values in the next count should be as follows:

♀ ♀ *yellow:* $14.21 \times 0.34 + 29 \times 0.66 = 24$ (%)

♀ ♀ *yellow-apricot:* $39.79 \times 0.34 + 25 \times 0.66 = 30$ (%)

♂ ♂ *yellow:* $7.75 \times 0.34 + 16 \times 0.66 = 13$ (%)

♂ ♂ *yellow-apricot:* $38.25 \times 0.34 + 30 \times 0.66 = 33$ (%)

From the above data, 44.4 per cent of females and 28.3 per cent of males are expected to have red-eyes (cf. count 3 in table 5). The values expected from the above calculations, as well as the observed values, are given in table 5 and graphically presented in figure 2. In this figure it is shown that the calculated and observed curves are similar, but that the observed number is always lower than the corresponding expected number.

Competition Between an Apricot-Eyed Fly and the Wild-Type Fly in the Population Cage

Materials

The *y-ap* strain used in the previous experiment was used in this experiment also. Females of this strain were mated with males of Hiroshima strain, and among their F_2 offspring, flies having the *y-ap*, +, *y*, or *ap* phenotypes were counted. The recombination value between *y* and *ap* genes was about 45 per cent and sex-ratio is approximately 1:1 ($\chi^2 = 1.585$; D.F. = 1; $0.2 < P < 0.3$).

Table 5. *Decreasing percentage of red-eyed flies in the population cage*

Count	Observed ♀ (y) %	Expected ♀ (y) %	Observed ♂ (y) %	Expected ♂ (y) %
1	100.0	100.0	0.0	0.0
2	55.2	53.7	12.5	34.8
3	26.6	44.4	9.0	28.3
4	16.6	37.0	5.7	23.9
5	12.9	29.6	3.2	19.6
6	6.5	24.1	2.4	17.4
7	3.2	20.4	1.8	15.2
8	1.2	16.7	1.1	13.0
9	1.2	13.0	0.3	10.9
10	0.5	11.1	0.2	8.7
11	0.2	9.3	0.0	6.5
12	0.2	7.4	0.0	6.5
13	0.0	5.6	0.0	4.3

Variations in number of flies in the population cage

This experiment was begun on January 1, 1950 and terminated on November 18, 1951. The population cage was started with seventy F_2 flies, i.e., thirty-five females of the genotype $\frac{y\text{-}ap}{y\text{-}ap^+}$ and thirty-five *y-ap* males. The cage was kept in the same room as the cage in the previous experiment, the flies were examined about once a month. The results are shown in table 6. The mean rate of substitution to the total number of flies is about 47 per cent. This rate shows considerable variation, besides a marked periodical drop at an interval of several counts.

Selective matings

The method used in this experiment was the same as that in Reed's experiment on *D. melanogaster*. Cultures in small vials were set up, each containing two virgin females and two males, of about 5 day age, of the genotypes ♀ ap^+/ap, ♀ ap/ap, ♂ ap/Y, ♂ ap^+/Y. These were placed together for 24 hours, then each female was transferred into a fresh vial and her offspring were examined.

If the females have apricot eyes and all her daughters have also apricot eyes, it is clear that apricot-eyed males mated with the females. If all the daughters have red eyes, they should have wild-type fathers. If there are both red- and apricot-eyed daughters in any ratio, both kinds of males have mated with the mother, i.e., double matings have occurred. If the female had red eyes and all her daughters have red eyes, her mate should be red-eyed males; if half of the daughters have red eyes and the other half apricot eyes, the father should be apricot-eyed. If both red- and apricot-

TABLE 6. *Number of living and dead flies in the population cage*

Date	Count	Living flies	Dead flies	Number of survivors	Number of substitution	Rate of substitution
Jan. 1, '50	1	70				
Jan. 25	2	504	17	53	451	0.89
Feb. 25	3	645	178	326	319	0.49
Mar. 24	4	595	259	386	209	0.35
Apr. 18	5	483	219	376	107	0.22
May 24	6	1070	158	325	587	0.64
Jun. 17	7	969	552	518	451	0.47
Jul. 12	8	582	562	407	175	0.30
Aug. 4	9	661	339	243	418	0.63
Sep. 1	10	547	286	375	172	0.31
Sep. 23	11	1182	105	442	740	0.63
Oct. 22	12	734	550	632	102	0.14
Nov. 18	13	1109	85	649	460	0.41
Dec. 13	14	1215	211	896	317	0.26
Jan. 8, '51	15	1040	282	933	107	0.10
Feb. 1	16	1082	369	671	411	0.38
Feb. 23	17	1197	227	855	342	0.29
Mar. 29	18	2231	496	701	1530	0.69
May 3	19	1237	1103	1128	109	0 09
Jun. 7	20	869	627	610	259	0.30
Jul. 7	21	1111	624	245	866	0.78
Aug. 1	22	1479	351	760	719	0.49
Aug. 23	23	1957	851	628	1329	0.68
Sep. 17	24	982	1093	864	118	0.12
Oct. 18	25	1811	616	366	1445	0.80
Nov. 18	26	2081	556	1255	826	0.40
Mean		1055.5			503	0.47

eyed daughters appear in any ratio, it is due to double matings. The results of this experiment are given in table 7. From the data in table 7, we can surmise that there were 114 red-eyed males (♂ ap^+/Y), which succeeded in mating, as against 71 apricot-eyed males (♂ *ap*/Y); the ratio is approximately 1.00:0.62 and the deviation from equality is statistically significant ($\chi^2 = 10.05$; D.F. = 1; $0.001 < P < 0.01$).

TABLE 7. *Results of the selective mating among red and apricot males and females*

	Mating of apricot ♀ (*ap*/*ap*)	Mating of red ♀ (*ap*/+)	Totals
apricot ♂	17	13	30
red ♂	29	44	73
Double mating	26	15	41
No offspring	10	14	24
Totals	82	86	168

Selective mating ratio
red ♂ : apricot ♂ (73+41) : (30+41) = 114:71
= 1.00:0.62

Competition leading to extinction

While this experiment was continued for about twenty-two months, the apricot-eyed flies gradually decreased in the population. The females, however, were always more numerous than males in the same population, the mean sex-ratio being about 62 ♀ : 38 ♂. The numbers of flies having different eye-colors are given in table 8.

As the thirty-five females, with which the experiment was started, had yellow body and red eyes, with the genotype $\frac{y\text{-}ap^+}{y\text{-}ap}$, and the thirty-five males had the genotype *y-ap*, an equal number of red-

TABLE 8. *Number of red-eyed and apricot-eyed flies*

Count	♀ red-eyed flies	♀ apricot-eyed flies	♂ red-eyed flies	♂ apricot-eyed flies
1	35	0	0	35
2	162	130	119	93
3	194	176	139	136
4	237	155	77	126
5	202	142	50	89
6	432	229	155	254
7	418	229	123	199
8	289	89	101	103
9	339	70	117	135
10	299	50	91	107
11	552	88	315	227
12	373	47	207	107
13	564	52	311	182
14	638	58	354	165
15	593	39	291	117
16	671	36	294	81
17	725	34	339	99
18	1230	52	735	214
19	851	31	266	89
20	702	29	121	17
21	635	25	362	89
22	843	36	482	118
23	1127	45	621	164
24	744	24	163	51
25	1022	41	630	118
26	1250	38	645	148
Total		♀ 17072	♂ 10371	
Sex-ratio		62 :	48	

and apricot-eyed offspring was expected in the next generation, thus:

♀ ♀	31% ap^+/ap	31% ap/ap
♂ ♂	19% ap^+/Y	19% ap/Y

The proportion of the eggs having the *ap* and ap^+ gene in the next generation is expected to be 0.93 (0.31 × 3):0.31.

As the ratio of success in selective mating in the previous section was 1.00 for red-eyed males as against 0.62 for apricot-eyed males, the expected proportion of the sperms having the *ap* and ap^+ gene would be 0.11 (0.19 × 0.62):0.19. The expected values of different kinds of female in the next generation are as follows:

	Eggs		
Sperms	0.19 ap^+ 0.11 ap	0.31 ap^+ 0.06 ap^+/ap^+ 0.03 ap^+/ap	0.93 ap 0.18 ap^+/ap 0.10 ap/ap

The frequency of each genotype may be estimated by the calculation and sex-ratio mentioned above:

♀ ap^+/ap^+:

$$62 \times \frac{0.06}{0.06+0.03+0.18+0.10} = 10.05$$

♀ ap^+/ap: $62 \times \frac{0.21}{0.37} = 35.19$

♀ ap/ap: $62 \times \frac{0.10}{0.37} = 16.76$

♂ ap^+/Y: $38 \times \frac{0.31}{0.93 + 0.31} = 9.50$

♂ ap/Y: $38 \times \frac{0.93}{1.24} = 28.50$

As to the number of living flies in this count, it is likely that the 53 per cent in the previous generation was added to the 47 per cent in the new generation, and the expected values in the next count should be as follows:

♀ ♀ *yellow* (ap^+/ap^+):
$10.05 \times 0.47 = 4.7 \doteqdot 5$ (%)

♀ ♀ *yellow* (ap^+/ap):
$35.19 \times 0.47 + 31.00 \times 0.53 = 32.9 \doteqdot 33$ (%)

♀ ♀ *yellow-apricot* (ap/ap):
$16.76 \times 0.47 + 31.00 \times 0.53 = 24.3 \doteqdot 24$ (%)

♂ ♂ *yellow* (ap^+/Y):
$9.50 \times 0.47 + 19.00 \times 0.53 = 14.5 \doteqdot 15$ (%)

♂ ♂ *yellow-apricot* (ap/Y):
$28.50 \times 0.47 + 19.00 \times 0.53 = 23.47 \doteqdot 23$ (%)

Thus, 61.3 per cent of females and 39.5 per cent of males are expected to have red eyes (cf. count 3 in table 9). The

TABLE 9. *Increasing percentage of red-eyed flies in the population cage*

Count	Observed ♀ (y) %	Expected ♀ (y) %	Observed ♂ (y) %	Expected ♂ (y) %
1	100.0	100.0	0.0	0.0
2	55.5	50.0	56.1	50.0
3	52.4	61.3	50.5	39.5
4	60.5	64.5	37.9	36.8
5	58.7	66.1	36.0	36.8
6	65.4	69.4	37.9	39.5
7	64.6	71.0	38.2	42.1
8	76.5	72.6	49.5	44.7
9	82.9	75.8	46.4	47.4
10	85.7	77.4	46.0	47.4
11	86.3	79.0	58.1	50.0
12	88.8	80.6	65.9	52.6
13	91.6	82.3	63.1	55.3
14	91.7	83.9	68.2	55.3
15	93.8	85.5	71.3	57.9
16	94.9	87.1	78.4	60.5
17	95.5	88.7	77.4	60.5
18	95.9	90.3	77.4	63.2
19	96.5	90.3	74.9	65.8
20	96.0	91.9	87.7	65.8
21	96.2	91.9	80.3	68.4
22	95.9	91.9	80.3	68.4
23	96.2	93.5	79.1	71.1
24	96.9	95.2	76.2	73.7
25	96.1	95.2	84.2	73.7
26	97.0	95.2	81.3	76.3

expected values estimated by the abovementioned calculations and the observed ones are summed up in table 9, and graphically presented in figure 3; the two curves are very similar in shape.

DISCUSSION

(1) The low fertility and viability of the hybrid males between *D. virilis* and *D. americana* has been considered to be a part of their reproductive isolation. This isolation seems to become more pronounced in the cage population. According to Koopman (1950), the hybrid between *D. pseudoobscura* and *D. persimilis* has lower adaptability to any available habitat than either parental type and its number in the population cage is kept down to only a few, so that the gene exchange between the parent species is largely prevented. This isolation is apparently strengthened in the laboratory population.

In the present experiment, the hybrids between *D. virilis* and *D. americana* coexisting with *D. virilis* gradually decreased in number, and were eventually eliminated completely from the laboratory population. This is apparently due

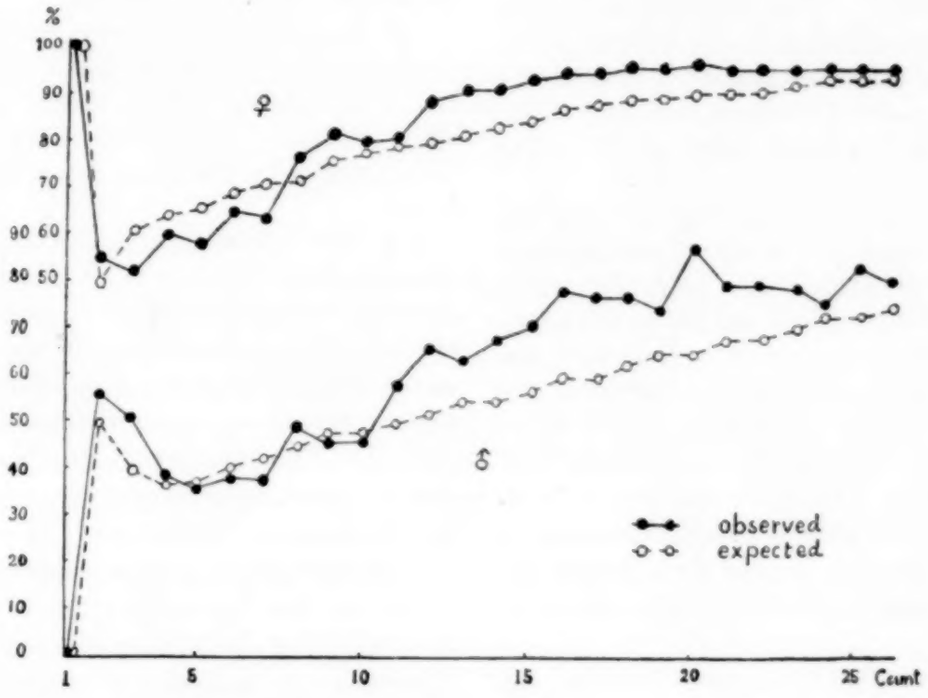

FIG. 3. Curves showing the increase of red-eyed flies.

primarily to the low fertility of hybrid males which is shown by the experiment of multiple choice by females. This low fertility may imply the immobility of the sperms, which is due to the immaturity of paragonia, as observed by Stone (1947). The hybrid males have been shown to be much inferior to the normal males in their mating ability during the first two weeks (table 2), and it is unlikely that they would be successful in mating in any crowded population.

Next, the remarkably high rate of secondary non-disjunction of sex-chromosomes of the hybrid females should be taken into consideration. This high rate is probably caused by the complex overlapping inversions in the X-chromosome and the fusion of X and fourth chromosomes in *D. americana*. It is likely that the rate of heterosynapsis between X- and Y-chromosomes of *D. virilis* in the reduction division is much higher than that of homosynapsis between the X-chromosomes of the different species. If so, the eggs containing the ap^+ gene located on *americana*'s X should be fewer than the eggs containing the *ap* gene located on *virilis*'s X, as the X-chromosome of *D. americana* will be eliminated in the meiosis. This assumption is supported by the fact that the hybrid males having the chromosome of *D. americana* are fewer in total number than the males having the X-chromosome of *D. virilis* (table 1).

The two curves, one representing the theoretical values calculated on the basis of these presumptions, and the other showing the observed values of the gradual change in number of hybrid flies, are rather similar (fig. 2). However, the former curve is invariably higher than the latter. This seems to indicate that the handicap factors against the hybrids are much strengthened in the population cage; or else the hybrid flies should encounter some unknown disadvantage in the cage.

(2) Reed (1950) observed in the experimental population of *D. melanogaster* that the flies having the sex-linked mutant white-eyes were inferior to normal red-eyed flies in various respects, and were eventually eliminated from the population. He thought that the most important factor for this difference was the difference in the ratio of selective mating between the two kinds of males.

My experiment with *D. virilis* on the selective mating between the apricot-eyed and the normal red-eyed males yielded a similar result (table 7). The theoretical values, calculated on the basis of this factor, the sex-ratio and of the rate of substitution in the population, has been found to coincide fairly well with the observed values (tables 8, 9, fig. 3).

Summary

(1) Hybrid flies having an X-chromosome of *D. virilis* and a complex X, of which the proximal part is derived from *D. americana* and the distal part of *D. virilis*'s X and autosomes of *D. virilis*, were placed in the same population cage with *D. virilis* carrying the sex-linked *apricot* gene. In about ten months, the hybrid flies decreased in number, and eventually they were eliminated completely from the population.

(2) In the ability for selective mating, the hybrid males were much inferior to *D. virilis* males. They could not find their mates in the crowded population. Their viability also was lower than the latter.

(3) The non-disjunctional rate of X-chromosome was very high in the hybrid females. *D. americana*'s X seems to be eliminated very frequently in the meiosis, as the gametes having this chromosome were much fewer than theoretically expected. The two curves illustrating the rate of decrease of hybrid flies, one showing the observed values and the other the calculated values, coincide fairly well.

(4) In the laboratory population of *D. virilis*, the proportion of the flies with apricot-eyes gradually decreased in competition with red-eyed flies. The ratio

of apricot-eyed males which succeeded in mating, compared with red-eyed males, was only 62 per cent. This is apparently one of the important causes for the gradual decrease of former in the population.

Acknowledgments

I wish to acknowledge my indebtedness to Dr. T. Komai for his kindness in the interpretation of the data and in the preparation of the manuscript. I am also grateful to Prof. H. Kikkawa and Prof. K. Nakamura for their help during the work.

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INDETERMINATE OUTCOME OF CERTAIN EXPERIMENTS ON *DROSOPHILA* POPULATIONS[1]

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INTRODUCTION

Repeatability of observations and experiments is taken for granted in science. If experiments on similar materials conducted under similar conditions fail to yield similar results, one suspects that some variables in the materials or in techniques have escaped detection. Yet, some natural processes happen only once; history, either on the biological or on the human level, does not repeat itself. There is, of course, nothing mysterious in this uniqueness. Evolutionary processes in nature are influenced by usually very numerous internal and environmental variables. It is unlikely that the whole constellation of variables can remain constant for long, or that it can recur in the future. The elementary components of the evolutionary process, the mutational and selectional steps, are both repeatable and reversible; evolution is however unrepeatable and irreversible. Geneticists and other experimentalists concentrate their attention chiefly on the elementary components, and are consequently reproached by those who study evolution by other means for not being able to observe evolutionary changes of any consequence. This reproach fails to take into account that historical events and trends become comprehensible only through understanding of the unspectacular everyday events which bring them about.

For several years, we have studied experimental populations of *Drosophila* which contain mixtures of chromosomal variants (reviews in Dobzhansky, 1947 and 1951). Genetic changes are observed in such populations, and most of these changes are reproducible within limits imposed by experimental errors. Recently, we have recorded also changes which appear to be of a different sort, in that they are reproducible only to a more limited extent. The present article reports the relevant data.

MATERIAL AND METHOD

All experiments were made with strains of *Drosophila pseudoobscura* descended from wild progenitors collected at Piñon Flats, Mount San Jacinto, California, and at Santa Barbara, Chihuahua, Mexico. The strains from Mexico were homozygous for the Chiricahua gene arrangement (CH), while some California strains homozygous for CH and others homozygous for the Standard gene arrangements (ST) were used. Each strain descended from a single fertilized female collected in the wild. The collections in Mexico were made by Professor H. T. Spieth in 1947, and in California by Mr. Alexander Sokoloff in 1945 and by Dr. D. F. Mitchell in 1949. The populations Nos. 66 and 68 were made with the older, and all other populations with the newer California strains (see tables 1–4). The same Mexican strains were used throughout.

The populations were kept in incubators at 25° C., in wooden population cages of the usual construction. Molasses-cream of wheat agarless medium with growing Fleischmann's yeast was used for food; when the jars showed signs of becoming too dry they were irrigated with an yeast suspension. The average length of the fly generation under these

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TABLE 1. *Percentages of standard chromosomes in two experiments involving mixtures of populations from California and from Mexico*

Population No. 66			Population No. 68		
Days	Generations	Percentage	Days	Generations	Percentage
0	0	50.0	0	0	50.0
30	1.20	57.0	30	1.20	59.7
60	1.20	63.7	75	1.80	69.0
100	1.60	72.7	125	2.00	79.7
140	1.60	76.7	155	1.20	82.3
180	1.60	71.0	200	1.80	88.7
220	1.60	72.0	260	2.40	95.3
310	3.60	69.3	320	2.40	96.7
360	2.00	64.3	—	—	—
420	2.40	69.0	—	—	—

conditions is about 25 days, although some flies hatch earlier and others later. The figure 25 days was used to compute the numbers of generations elapsed between successive samples (tables 1 and 4).

The populations were sampled about once a month at the beginning, and less often at the later stages. Each sample consisted of 300 chromosomes, obtained from 150 larvae, taken in 6 subsamples on six successive days, 25 larvae (50 chromosomes) per subsample. The chromosome frequencies in tables 1–4 are, thus, based on 300 observations each, unless otherwise specified. The day when the third subsample was taken was considered the date of the whole sample.

THE EARLY EXPERIMENTS

Experimental populations which contain two kinds of chromosomes, with different gene arrangements but derived from the same natural population, present the simplest situation which is easily analyzed and understood. In such populations, the structural heterozygotes are usually superior in adaptive value to both homozygotes. Because of this heterosis, the process of natural selection leads, in experimental and also in natural populations, to the establishment of equilibria, at which the chromosomes of different kinds continue to occur with definite frequencies (Dobzhansky, 1947, 1951). Only a single exception to this rule has so far been found; in this exceptional case a gene arrangement which is rare in a natural population showed heterosis with some, but not with all, chromosome types which occur in that population (Dobzhansky, 1948).

Chromosomes with the same gene arrangement derived from different geographic populations may contain unlike gene complexes. This is shown by variations in the equilibrium points attained in experimental populations derived from different localities, even localities only a dozen or so miles apart (Dobzhansky, 1948). No simple rule describes the behavior of experimental populations in which the two kinds of chromosomes are of different geographic origin. Some populations of such mixed origin reach equilibria, at which the gene arrangements coexist seemingly indefinitely. In populations which reach equilibria, the structural heterozygotes evidently exhibit heterosis, at least when the equilibrium is reached. But other mixed populations fail to attain equilibria, and one of the competing chromosome types is eventually eliminated entirely while the other reaches fixation. This means that heterosis is absent, and the structural heterozygotes are equal or inferior to one of the homozygotes in adaptive value (Dobzhansky, 1950).

A most remarkable population has been described by Dobzhansky and

Levene (1951). The foundation stock of this population consisted of F_1 hybrids between ST strains from California and CH strains from Mexico. The frequencies of ST and CH chromosomes were, accordingly, equal, and amounted to 0.50 (50 per cent). This population (No. 66, table 1) was started on November 15, 1949. It was known previously that, among the F_2 California × Mexico hybrids, the ST/ST homozygotes were more viable than the ST/CH heterozygotes, and the latter were more viable than the CH/CH homozygotes (Dobzhansky and Levene, 1951). This suggested that heterosis was absent, and led to the expectation that in the population No. 66 the CH chromosomes will eventually be eliminated, and ST will reach fixation.

Indeed, for some months the frequencies of ST chromosomes increased rapidly, and in early April, 1950 (about 140 days after the start) reached 76.7 per cent (table 1 and fig. 1). But thereafter the ST chromosomes not only failed to increase further, but in fact dropped to about 69 per cent frequency level, at which an equilibrium became established. This equilibrium was maintained for about six months, until mid-January of 1951, when the population was terminated. A test conducted in October 1950 revealed the fact that at that time the ST/CH heterozygotes from population No. 66 were more viable than the ST/ST homozygotes, and these latter were more viable than the CH/CH homozygotes. It appears, then, that heterosis has developed in this population, although it was not present there at the beginning of the experiment.

A statistical analysis by Levene (Dobzhansky and Levene, 1951) confirmed the above interpretation. It showed that the adaptive values of ST/CH, ST/ST, and CH/CH chromosomal types underwent changes in population No. 66. The behavior of the population for the first 5–6 generations (about 140 days, table 1) is compatible with the assumption that the adaptive values for the three genotypes were about 1, 1, and 0.5 respectively. Thereafter the best fit is obtained with values 1, 0.65, 0.3 respectively. Of course, this does not mean that heterosis developed suddenly some 140 days after the beginning of the experiment. It is more likely that the heterotic condition arose gradually during that period.

The First Replication

The extraordinary result obtained suggests that in population No. 66 a heterotic state has developed, presumably under the influence of natural selection.

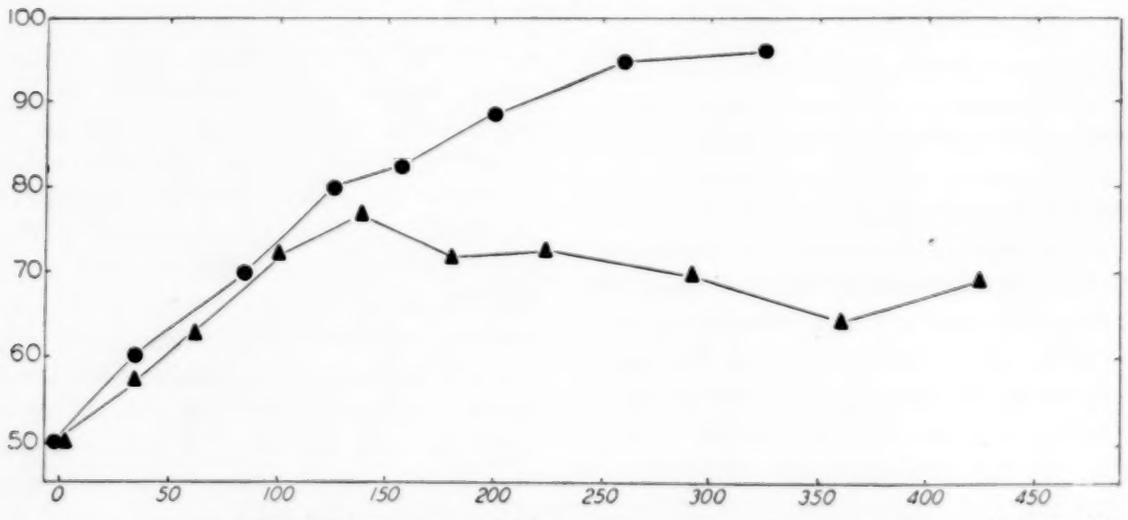

Fig. 1. Frequencies of chromosomes with the ST gene arrangement in populations No. 66 (triangles) and No. 68 (circles). Vertical axis—per cent ST chromosomes, horizontal axis—time in days.

TABLE 2. *Percentages of standard chromosomes in replicate experiments on populations of geographically pure and geographically mixed origins*

	Geographically pure					Geographically mixed			
Days	90	91	92	96	Mean	89	93	94	95
0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
35	37.7	37.3	38.3	32.0	36.3	32.8	31.3	31.3	27.0
80	50.0	59.0	52.7	52.0	53.4	58.7	55.3	57.0	55.0
120	63.0	70.0	60.7	61.7	63.8	68.3	58.0	85.3	71.0
160	69.0	71.3	68.7	66.0	68.8	76.0	63.3	90.7	79.3
200	74.7	76.0	69.0	76.3	74.0	80.7	72.0	97.3	86.0
250	78.0	80.0	80.7	79.0	79.4	86.0	91.7	98.3	92.0
310	78.7	84.0	80.3	83.0	81.5	81.0	97.7	97.0	96.7
365	83.0	84.0	80.7	80.3	82.0	80.0	99.0	—	97.7

This would be expected to occur if heterosis brought an increase of the mean adaptive value of the population as a whole. The foundation stock in population No. 66 was a mixture of twelve strains from Mexico and fifteen from California. If some, perhaps only one or two, of the chromosomes introduced with these strains had heterotic properties, these chromosomes would become multiplied by natural selection at the expense of the non-heterotic genotypes.

The experiment had obviously to be repeated, and on October 31, 1950, population No. 68 was started, using exactly the same California and Mexico ST and CH strains which were used for the foundation stock of population No. 66. The course of the events in population No. 68 is shown in table 1 and figure 1. For some 100 days (4 generations) the changes were almost exactly like those in population No. 66. But thereafter the frequency of ST chromosomes continued to rise. It reached about 89 per cent in 200 days and about 97 per cent in 320 days, when the experiment was terminated. There is, of course, no doubt that the outcomes in populations Nos. 66 and 68 are significantly different. No heterosis has developed in No. 68, and the CH chromosomes in this population should, if given enough time, be eliminated entirely. In practice, it is not expedient to continue the experiments after the less frequent gene arrangement falls below about the 5 per cent level. With samples consisting of 300 chromosomes, the sampling errors become relatively large, and the changes become so slow that the labor expended on the maintenance of the populations is not justified.

REPRODUCIBILITY OF THE RESULTS IN POPULATIONS OF GEOGRAPHICALLY PURE ORIGIN

The failure of the replicate experiments Nos. 66 and 68 to give similar results may be due to the foundation stocks in these populations having consisted of interracial hybrids. The races of *D. pseudoobscura* which inhabit California and Mexico may differ in many genes, and the F_2 generation from their cross (which is the first generation developing in the population cages) may contain a very great variety of genotypes. Because the population is finite in size, some of the genotypes which are potentially possible in such a population may or may not actually be formed. This may affect the outcome of the experiments. The situation should, then, be different in experimental populations in which the progenitors came from the same or from different natural populations. Although all experimental populations are made by mixing as many strains derived from different wild progenitors as possible, the genetic diversity is likely to be very much lower when the parent strains are of geographically uniform than when they are of geographically mixed origin.

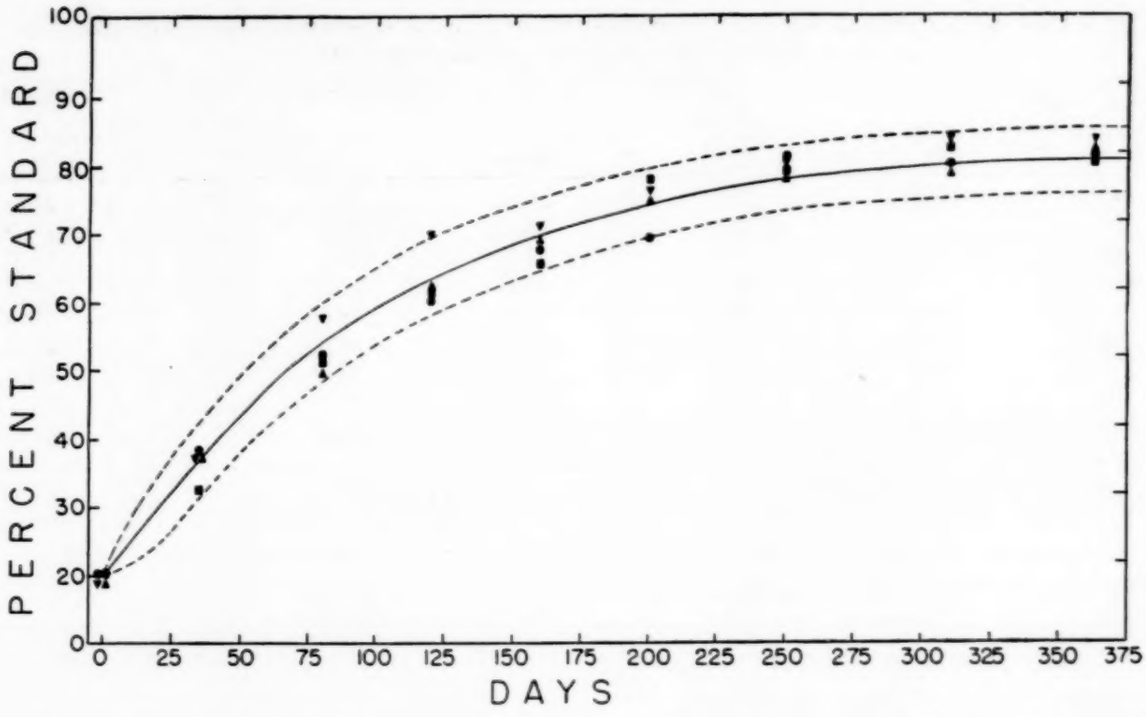

FIG. 2. Frequencies of chromosomes with the ST gene arrangement in populations of geographically uniform origin. Population No. 90—triangles upwards, No. 91—triangles downwards, No. 92—circles, No. 96—squares. Solid curve—frequencies of ST chromosomes expected with adaptive values of ST/ST : ST/CH : CH/CH genotypes being 0.89 : 1 : 0.41 respectively. Dashed curves indicate the 95 per cent confidence limits for the expected values of ST.

Seven experimental populations with ST and CH chromosomes from California (the Piñon Flats locality) have been described in previous publications (Dobzhansky, 1947a, b, 1948; Wright and Dobzhansky, 1946). All of these populations were run at 25° C., or at room temperatures slightly below that, but otherwise they were not strictly speaking replications. They were made at different times, the foundation stocks had different initial frequencies of the chromosomal types, some were run in normally lighted rooms, and others in the dark, etc. It is, therefore, significant that all seven populations have reached, or apparently tended to reach, equilibria at the levels of 70–75 per cent of ST and 25–30 per cent of CH chromosomes. In other words, the ST/CH heterozygotes in these populations exhibited heterosis. A more rigid test of the uniformity of the behavior of replicate populations was made as follows.

On November 14 and 15, 1951, three populations (Nos. 90, 91, and 92) were started with 20 per cent ST and 80 per cent CH chromosomes of California (Piñon Flats) origin. On December 31, 1951, a fourth population (No. 96) of the same kind was added. In each population, the foundation stock consisted of 900 homozygous CH/CH and 600 heterozygous ST/CH flies. Both kinds of flies were F_1 hybrids of the twelve CH and twelve ST strains received from Dr. D. F. Mitchell (see the section on Material and Method). The four populations were, thus, replicates of each other, but they descended from different progenitors from the seven populations mentioned above, the ancestors of which were collected earlier in the same locality by Mr. A. Sokoloff.

The populations were sampled 35, 80, 120, 160, 250, 310, and 365 days after the start. The results are shown in tables 2 and 4 and figure 2. In all popu-

lations the frequencies of ST rose rapidly at first, more slowly thereafter, and reached close to the 80 per cent level of ST by about 250 days. Little increase occurred thereafter, and the populations were terminated when one year old. The attainment of the equilibrium shows that the ST/CH heterozygotes exhibited heterosis in all populations. To test the homogeneity of the results in the four populations, chi-squares were computed for the groups of corresponding samples. As shown in table 3, the degree of heterogeneity observed has as low as 0.07 probability of being due to sampling errors in only a single case—that of the 120-days samples. It is, therefore, justified to pool the data for the four populations. The mean frequencies of ST chromosomes are reported in table 2.

To analyze the process of selection in more detail, the method suggested by Levene (in Dobzhansky and Levene, 1951) was used. Table 4 gives the figures for the numbers of generations elapsed between the successive samples (t), the ratios of the frequencies of CH and ST chromosomes (x), the standard errors of these frequencies, and the regression slopes (r), computed from the formula $r = (X_1/X_0)^{1/t}$. It can be seen that the values of r gradually increase from 0.56 at the beginning, to close to 1, indicating no further change, at the end of the experiment. Taking the adaptive value of the heterozygotes to be unity, an attempt may be made to find the adaptive values of the CH/CH homozygotes (W_1) and the ST/ST homozygotes (W_2). These values may be found graphically, by plotting the linear equations for the time intervals between the different samples, as shown in the upper left corner of figure 3, and finding the points of intersection. In this and other diagrams in figures 3, and 4, the lines are lettered as follows:

Interval, days	Letter	Interval, days	Letter
0– 35	A	160–200	E
35– 80	B	200–250	F
80–120	C	250–310	G
120–160	D	310–365	H

It can be seen in figure 3 that most of the lines intersect at values of W_1 between 0.25 and 0.50, and at values of W_2 between 0.85 and 0.95. These variations are well within the limits of sampling errors. Only the lines F, G, and H intersect at values of W_1 below 0, which is biologically impossible, since a negative W would mean that carriers of a certain genotype suffer a fate worse than death. A glance at table 4 shows, however, that these meaningless results are obtained when the populations approach the equilibrium, and the net changes in the chromosome frequencies between the successive samples do not greatly exceed their sampling errors. Lines G and H, and in most cases also F, are accordingly omitted in the diagrams in figures 3 and 4.

TABLE 3. *Uniformity or heterogeneity of the outcomes in replicate experiments on geographically pure and mixed populations*

Every chi-square has three degrees of freedom

	Geographically pure		Geographically mixed	
Days	Chi-Square	P	Chi-Square	P
35	3.26	0.35	1.15	0.75
80	5.48	0.15	1.06	0.80
120	6.95	0.07	55.15	<0.001
160	2.00	0.55	64.96	<0.001
200	5.43	0.15	75.19	<0.001
250	0.76	0.85	31.06	<0.001
310	3.55	0.30	89.96	<0.001
365	1.95	0.55	93.12	<0.001

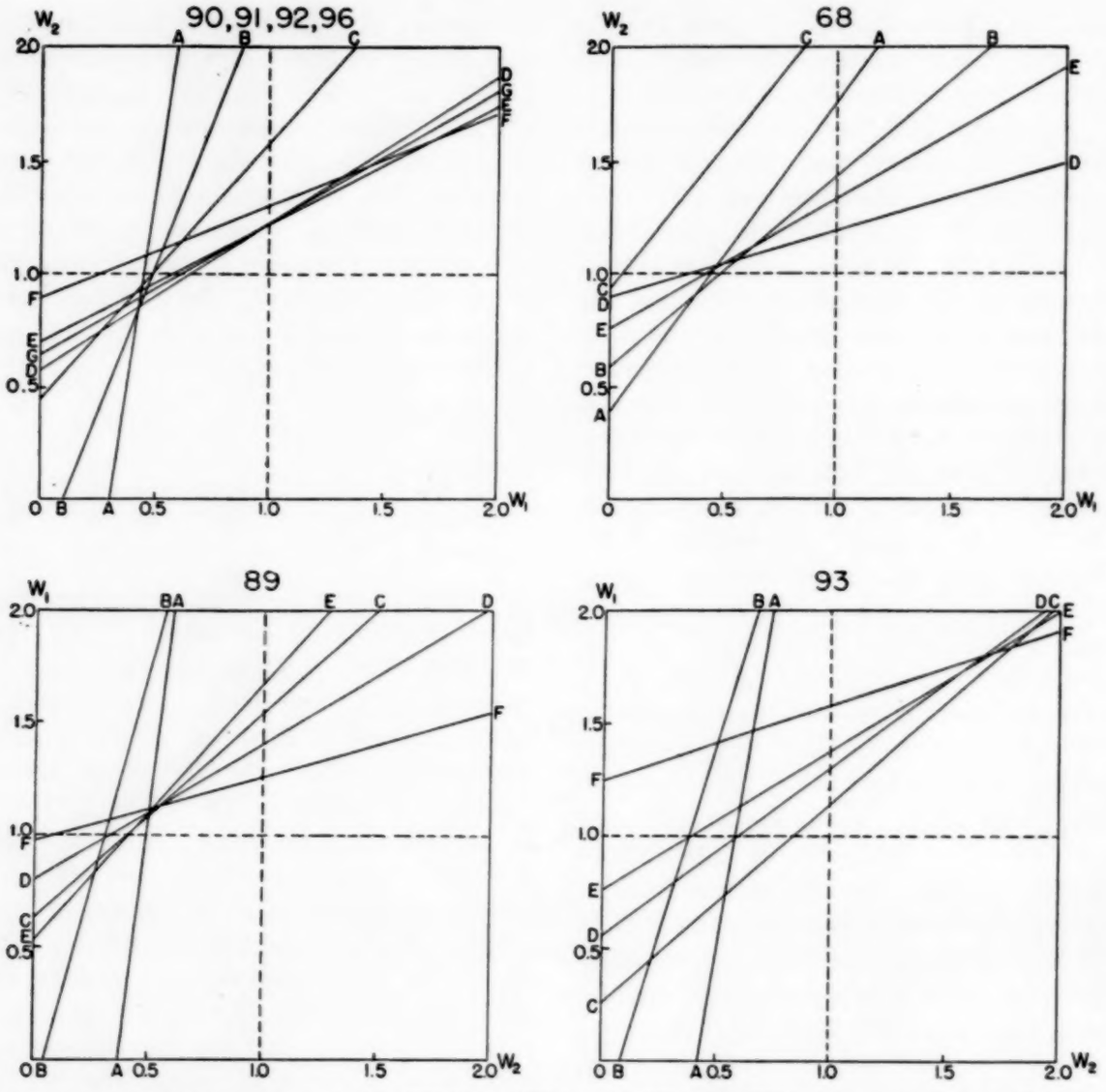

FIG. 3. Adaptive values of the CH/CH (W_1) and ST/ST (W_2) homozygotes at different times in the experimental populations. A, B, C, D, E, and F are the time intervals from the beginning to the end of the experiments. The points of intersection of these lines indicate the adaptive values.

The evidence is consistent with the assumption that the adaptive values W_1 and W_2 remained constant during the course of the experiments in the populations 90, 91, 92, and 96. It is therefore justified to estimate the values of W_1 and W_2 for the entire series of experiments, using the least square method of Wright and Dobzhansky (1946). The estimates turn out to be:

Genotype	ST/ST	ST/CH	CH/CH
Adaptive Value	0.895	1	0.413

The selection coefficients against the homozygotes, S_1 and S_2 are respectively $1-W_1$ and $1-W_2$, or 0.105 for ST/ST and 0.587 for CH/CH. The populations are expected to reach an equilibrium when the frequency of Standard chromosomes becomes equal to $S_2/(S_1 + S_2)$, which is in our case 0.848, or about 85 per cent. This agrees reasonably well with the experimentally obtained value: after a year of operation of natural selection the mean frequency of ST chromosomes in the populations Nos. 90, 91, 92, and 96 is 82.0 ± 1.11 per cent. This is only slightly below the expected equilibrium value.

The solid black line in figure 2 shows

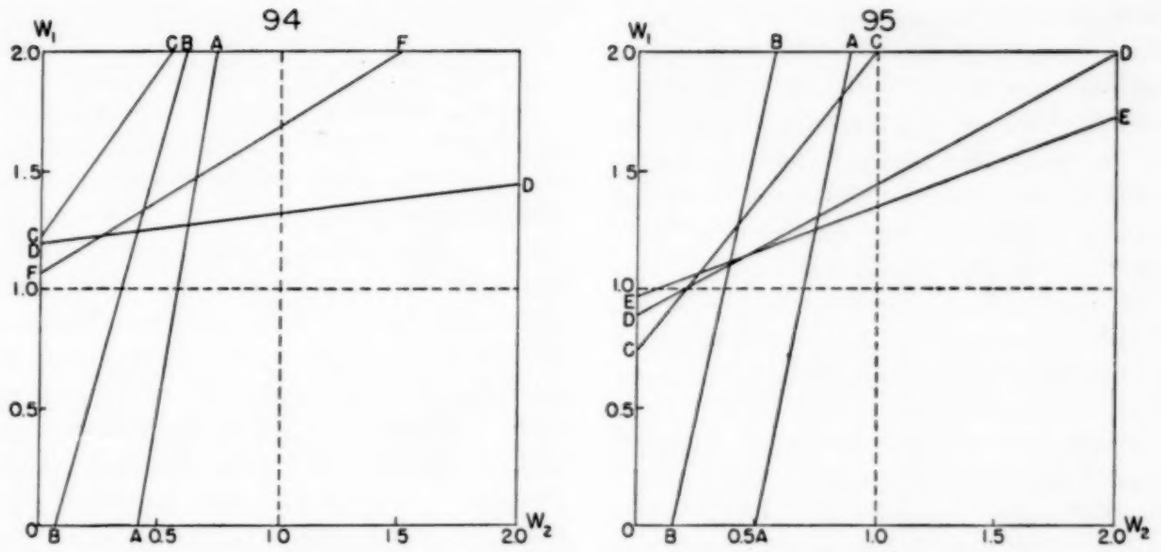

FIG. 4. The adaptive values of the CH/CH (W_1) and ST/ST (W_2) homozygotes in the populations Nos. 94 and 95. The interpretation of the lines same as in figure 3.

TABLE 4. *Analysis of the changes observed in experimental populations, leading to determination of the adaptive values of the genotypes*

t—the time interval in generations; x—ratio of the frequencies of CH and ST chromosomes; r—adjusted change in x per generation

Days	t	x	r	x	r
		Population No. 89		Population No. 93	
0	—	4.000	—	4.000	—
35	1.44	2.049 ± 0.252	0.628	2.195 ± 0.272	0.660
80	1.76	0.704 ± 0.082	0.541	0.808 ± 0.094	0.563
120	1.60	0.449 ± 0.055	0.755	0.724 ± 0.085	0.934
160	1.60	0.316 ± 0.043	0.802	0.580 ± 0.069	0.871
200	1.60	0.239 ± 0.035	0.840	0.389 ± 0.050	0.779
250	2.00	0.163 ± 0.027	0.826	0.091 ± 0.019	0.484
310	2.40	0.235 ± 0.035	1.116	0.024 ± 0.010	0.574
360	2.20	0.250 ± 0.036	1.102	0.010 ± 0.006	0.672
		Population No. 94		Population No. 95	
0	—	4.000	—	4.000	—
35	1.44	2.195 ± 0.272	0.660	2.704 ± 0.341	0.761
80	1.76	0.754 ± 0.088	0.541	0.818 ± 0.094	0.503
120	1.60	0.172 ± 0.028	0.397	0.408 ± 0.052	0.647
160	1.60	0.103 ± 0.020	0.726	0.261 ± 0.036	0.757
200	1.60	0.028 ± 0.010	0.442	0.163 ± 0.027	0.746
250	2.00	0.017 ± 0.009	0.779	0.087 ± 0.018	0.731
310	2.40	0.031 ± 0.010	1.285	0.034 ± 0.011	0.676
360	2.20	— —	—	0.024 ± 0.009	0.854
		Populations Nos. 90, 91, 92, 96		Population No. 68	
0	—	4.000	—	1.000	—
35	1.44	1.755 ± 0.105	0.564	0.675 ± 0.251	0.722
80	1.76	0.873 ± 0.051	0.672	0.449 ± 0.055	0.796
120	1.60	0.567 ± 0.033	0.764	0.255 ± 0.036	0.708
160	1.60	0.455 ± 0.028	0.872	0.215 ± 0.033	0.868
200	1.60	0.351 ± 0.023	0.850	0.127 ± 0.023	0.745
250	2.00	0.259 ± 0.019	0.859	0.049 ± 0.013	0.670
310	2.40	0.227 ± 0.017	0.947	0.034 ± 0.011	0.857
365	2.20	0.220 ± 0.016	0.986	— —	—

the expected frequencies of ST chromosomes, computed on the basis of the above adaptive values. The dotted lines enclose the 95 per cent confidence band. It can be seen that among the 32 experimentally determined values, only one lies slightly outside of, and one on the outer boundary of the confidence band. This is as good a fit as can reasonably be expected. The outcome of natural selection in populations of geographically uniform origin is determinate.

It may, however, be noted that the older experiments on competition of ST and CH chromosomes of Piñon, California, origin yielded somewhat different adaptive values, namely 0.77 to 0.85 for ST/ST and 0.58 to 0.61 for CH/CH (Dobzhansky, 1948). The equilibrium in these experiments was between 70 and 75 per cent. The reason for this discrepancy is unclear. Involuntary changes in technique may be involved. More likely, the difference is due to employment of a different set of strains for the foundation stocks in the newer experiments.

Divergence of Populations of Geographically Mixed Origin

Four replicate populations of geographically mixed origin were run simultaneously with the four replicates of geographically uniform origin described above. The mixed populations Nos. 89, 93, and 94 were started on November 15th, and No. 95 was added on December 22, 1951. Each population contained initially 20 per cent ST chromosomes from California (Piñon), and 80 per cent CH chromosomes from Mexico (Chihuahua). The foundation stocks in the populations consisted of 900 CH/CH and 600 CH/ST flies. The twelve CH strains from Mexico were crossed to each other and to the twelve ST strains from California, and the F_1 hybrids were used to make the foundation stocks. The results are reported in tables 2 and 4, and in figures 3, 4, and 5.

The populations were sampled at the same time as the geographically uniform ones. The samples made 35 and 80 days after the start showed that the frequencies of ST chromosomes were rapidly

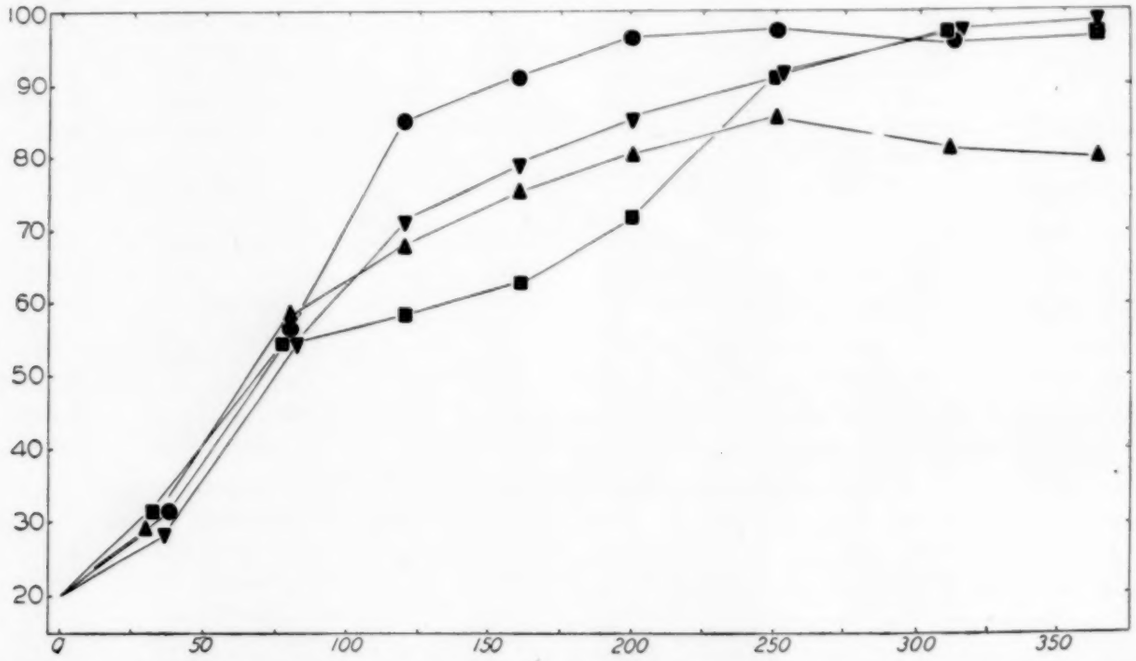

Fig. 5. Frequencies of chromosomes with the ST gene arrangement in populations descended from hybrids between California and Mexican strains. Population No. 89—triangles upwards, No. 93—squares, No. 94—circles, No. 95—triangles downwards. Vertical axis—per cent ST chromosomes, horizontal axis—time in days.

rising in all populations. Furthermore, the rise in all populations was uniform. But the samples at the age of 120 days disclosed a divergence of the different populations. Population No. 93 contained about 58 per cent ST chromosomes, while population No. 94 had as much as 85 per cent. Nos. 89 and 95 were intermediate (figure 5). The frequency of ST in No. 94 rose to 97 per cent at the age of 200 days; No. 93 progressed much less rapidly, but at the age of 310 days it reached the same level as No. 94. Nos. 89 and 95 eventually diverged also, No. 95 losing almost all CH chromosomes by the time it was one year old, while No. 89 established an equilibrium at the level of about 80 per cent ST and 20 per cent CH chromosomes. In short, no two of the replicate populations gave similar results (figure 5).

The chi-squares for the replicate populations are shown in table 3. No significant heterogeneity was present at the ages of 35 and 80 days, but thereafter the heterogeneity was highly significant. Moreover, the results were not only quantitatively but also qualitatively heterogeneous. The equilibria reached in populations Nos. 66 and 89 indicate that, in these populations, the heterozygotes ST/CH became heterotic, i.e., superior in adaptive value to the homozygotes. No heterosis has evolved in the other populations, Nos. 68, 93, 94, and 95. In these populations the adaptive values of the chromosomal types form the series ST/ST > ST/CH > CH/CH.

Figure 5 shows that the four replicate populations were alike to begin with, but that differences appeared between the ages of 80 and 120 days. This suggests that the adaptive values of the chromosomal types suffered changes in the course of the experiments. The method of analysis of Wright and Dobzhansky (1946) is consequently inapplicable, and that of Dobzhansky and Levene (1951) must be used instead. Table 4 shows the pertinent data (for the population No. 68, the days of sampling and the numbers of generations are shown in table 1). The diagrams in figures 3 and 4 show the lines the intersections of which indicate the changing adaptive values of CH/CH (W_1) and ST/ST (W_2). Different results are observed in different populations.

The situation is perhaps simplest in population No. 68. Most of the lines intersect at values of W_1 in the vicinity of 0.5, and of W_2 slightly above 1.0. This means that there is no heterosis, and ST/ST homozygotes are superior to ST/CH heterozygotes. Only the lines C and D intersect at a value of W_1 below 0, which is clearly a matter of sampling errors. There is no suggestion that the adaptive values underwent changes in this population.

In populations Nos. 89, 93, 94, and 95, the intersections of the lines A and B indicate very high values of W_2, i.e., a great superiority of ST/ST homozygotes at the beginning of the experiments. But the intersections of B with C, C with D, etc, indicate values of W_2 in the vicinity of 1, although most of them are still above unity. Although the sampling errors (table 4) would clearly vitiate any attempt to follow the changes of the adaptive values in the individual experiments, it is no doubt justified to conclude that they have not remained constant, and that in at least population No. 89 they tended towards establishment of a heterotic condition.

Discussion

We have studied experimental populations of *Drosophila pseudoobscura,* which contained mixtures of chromosomes with different gene arrangements. In some of these populations all the chromosomes were derived from wild progenitors collected in a single geographic locality; in other populations the progenitors were interracial hybrids, descended from flies collected in California and in Mexico. The outcomes observed in populations of geographically uniform and in those of geographically mixed

origins were strikingly different. In the former populations the replicate experiments yielded similar results, within limits of sampling errors. In the latter populations replicate experiments brought very significantly divergent results. In the populations of geographically uniform origin, the structural heterozygotes which carried chromosomes with different gene arrangements exhibited heterosis. Heterosis was observed in some but not in other populations of mixed origin. In such populations, heterosis is usually absent at the beginning of the experiments, and it may or may not develop with time.

It may now be inquired, why are the outcomes so constant in the replicate experiments which involve populations of geographically uniform origin, and so variable in populations of mixed origin? The possibility that undetected environmental differences are responsible should be given consideration. This possibility must, however, be rejected. Indeed, the experiments of the two kinds were run simultaneously, and the environmental differences between populations of mixed origin were no greater than those between the replicates of geographically uniform origin. A more fruitful hypothesis is that the indeterminacy is, in our experiments, a consequence of hybridization of strains of geographically remote origin.

Any natural population of *Drosophila* consists of individuals which carry a variety of genotypes. The foundation stocks of our experimental populations always included more than twenty different strains, thus insuring the presence of genetic variance in all populations. There is no way at present to estimate exactly how much greater this variance was in populations of geographically mixed than in those of geographically uniform origin, but the disparity was certainly quite considerable. The foundation stocks in mixed populations were F_1 hybrids of Mexican and California strains. The genetic segregation in F_2 generation of this cross may have amounted to what could be called a "genetic explosion." In this and the following generations the segregation products were, in all experiments, exposed to the action of natural selection. The genetic materials with which the selection processes operated were however not the same in populations of uniform and in those of mixed origin. In the former, the chromosomes with different gene arrangements carried gene complexes coadapted in the evolutionary history of the ancestral population to give heterosis in the inversion heterozygotes. The genetic background (i.e., the gene complexes in the chromosomes other than the third which carried the inversions) was also adjusted to make the heterotic mechanisms function as smoothly as possible. The selection in experimental populations of geographically uniform origin changed only the frequencies of pre-existing genetic variants. These changes led to the establishment of the equilibrium frequencies of the different chromosomes which were present in the foundation stocks of the populations. They were microevolutionary changes.

In the populations of mixed origin the changes were more profound. The numerous genotypes which arose in the offspring of the race hybrids evidently had different adaptive values under experimental conditions. Natural selection tended, therefore, to perpetuate the gene combinations which were adaptively superior, and to reduce the frequencies of the inferior ones. Since heterosis maximizes the average adaptive values of populations as wholes, heterotic genotypes were selected in some experiments. But in other populations no heterotic mechanisms became established, either because the heterotic genotypes did not happen to be produced, or because they arose in chromosomes other than the third, which, in our experiments, was marked by a cytologically visible inversion. When a population contains a large number of unfixed genes, the number of potentially possible gene combina-

tions may be very great. Which ones of these genotypes will be produced will, in finite populations, be a matter of chance. The efficiency of natural selection being also limited, some adaptively valuable gene combinations may be lost, even if they do arise. The result of these uncertainties will be that the outcome of natural selection will not be necessarily uniform, even in populations which are genetically similar to begin with.

The variety of genotypes which may arise in the offspring of race hybrids may be so great that evolutionary changes of considerable extent and complexity may occur without new genotypes arising by mutation (although, on the other hand, we can not exclude the possibility that mutation intervened in some populations). However that may be, the natural selection in populations of mixed origin resulted in emergence of new, and adaptively coherent, genotypes which were not present in the original materials used for the foundation stocks. Even though these new adaptive genotypes were compounded from pre-existing genetic elements, the changes observed in populations of mixed origin surpass the scale of the changes observed in populations of geographically uniform origin. In the former, new adaptive genotypes arose and became established, while in the latter the alterations involved chiefly the relative frequencies of certain previously available genotypes. If the latter changes are labelled microevolution, the former may perhaps be referred to as mesoevolution. The words macroevolution and megaevolution would, then, be reserved to designate emergence of new types of body structure and function, requiring time in geological scale, and usually leading to formation of new genera, families, and still higher categories. Needless to say, these four words refer only to the dimensions of the changes which they describe, while most evolutionists regard it as probable that the essential genetic mechanisms are similar throughout. Furthermore, one may expect to find all transitions between microevolutionary and megaevolutionary changes. We believe, nevertheless, that the above words may be useful to convey ideas concerning the scale of the evolutionary changes described in a given case. Thus in our experiments, the microevolutionary changes proved fully reproducible and repeatable, while the mesoevolutionary ones were repeatable to only a limited extent.

Acknowledgments

The experiments described in this paper were conducted for a period of about three years, during which time constant attention was required for their maintenance. Mr. and Mrs. B. Spassky have repeatedly shared with us this work. Mr. Louis Levine has made the determinations of the gene arrangements in the chromosomes during the summer of 1952, when the senior author was engaged in field work in Brazil. Prof. Howard Levene and Prof. Sewall Wright have given invaluable advice concerning the statistical methods. The interpretation of the results has been discussed with many colleagues in the United States and abroad, and these discussions have helped us greatly to clarify our ideas concerning the relations between microevolutionary and mesoevolutionary changes.

Summary

Experimental populations of *Drosophila pseudoobscura* were made which contained mixtures of flies with ST and with CH gene arrangements in their third chromosomes. In some populations all chromosomes were of geographically uniform origin, deriving from wild ancestors collected in a certain locality in California. In other populations the flies of the foundation stock were race hybrids, the carriers of the ST chromosomes descending from California, and those of the CH chromosomes derived from ancestors collected in Mexico.

The outcomes of the replicate experiments on population of geographically uni-

form origin were alike, within the limits of sampling errors. The heterozygotes ST/CH exhibited heterosis, and the populations eventually reached equilibria, with ST and CH chromosomes continuing to occur with predictable frequencies.

Replicate populations of geographically mixed origin exhibited a variety of behaviors. The ST/CH heterozygotes in such populations originally possessed no heterosis. But in two out of the six populations heterosis has eventually developed, and the populations reached equilibrium. In four remaining populations heterosis did not develop, and CH chromosomes tended to be eliminated completely. The adaptive values of the ST/ST, ST/CH, and CH/CH genotypes changed with time. In fact, no two of the populations of mixed origin were quite alike.

The changes induced by natural selection in populations of geographically mixed origin are more profound than those which occurred in populations of geographically uniform origin. In the former, new adaptively coherent genotypes have been molded by natural selection from the segregation products of the interracial cross. In the latter, only changes in frequencies of preexisting genetic variants have taken place. The former can be referred to as mesoevolutionary and the latter as microevolutionary changes. The predictability and repeatability of mesoevolutionary changes in finite populations are limited.

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THE ORGANISM AND ITS ORIGIN

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INTRODUCTION

"What is life?", "What is an organism?" and "How did life originate?" are questions of fundamental importance. Answers to these questions form the basis of the subject matter of biology; and until man discovers these solutions, his knowledge of biology must remain fragmentary and unsatisfying. To assert that the solutions of these problems will resolve all questions in biology is probably false; however, if biology is a science distinct from chemistry and physics, and if biological phenomena are based on chemical and physical phenomena, then presumably a group of assumptions, definitions and principles exist which unify biology and set it apart from all other science. That this set of definitions and assumptions is intimately related to these questions seems highly probable. The purpose of this paper is to investigate this probability.

Answers to the first question have been proposed by but a few men in recent times. For example, Osborn (1917) proposes that life depends in part on the activities of the developing organism and its hereditary material and in part on the environment. This view later became the organismal theory of life. Calkins (1933) states that "life may be defined as *protoplasmic organization manifesting vitality or a potential of vitality*" and defines "vitality, therefore, *as the sum total of actions, reactions and interactions between and amongst the substances making up the organization of protoplasm and between these and the environment*" (p. 47, italics his). These definitions are cyclic and may be restated as: life is protoplasm behaving as protoplasm.

Many biologists consider life too complex to define and substitute for a definition a list of characteristics common to all organisms. The use of such a list is unsatisfactory because (1) non-living systems frequently exhibit one or more of these characters, (2) seldom, if ever, does an organism manifest all of these attributes at one time, and (3) the characters can be demonstrated in some organisms, such as the viruses and some bacteria, with difficulty.

Both Troland and Alexander and Bridges (Alexander, 1948) consider the attributes of life; and Alexander asserts: "The essential criteria of life are twofold: (1) the ability to direct chemical change by catalysis; (2) the ability to reproduce by autocatalysis" (p. 88). Although "the essential criteria of life" appear to solve the problem of what life is in a very neat and simple manner, closer examination of them raises serious questions. Systems of purified enzymes and substrates can be set up which satisfy the first criteria but not the second; are these alive? If the answer is no, then is life synonomous with the second criterion?

Other workers propose answers to the question, "What is life?", which depend upon the organism as a whole, organization, vitalism, or purpose (Lillie, 1945; Sommerhoff, 1950; Sinnott, 1950); or even suggest that life is subject to a different set of physical laws than is nonliving matter (Schrödinger, 1947). However, not one of these suggestions has been developed to serve as a basis for the field of biology.

Pirie (1937), while considering the qualities to define the word *life*, finds that "they are individually inadequate for even an approximate definition . . ." and proposes that "until a valid definition has been framed it seems prudent to avoid the use of the word 'life' in any discus-

sion about border-line systems and to refrain from saying that certain observations on a system have proved that it is or is not 'alive'" (p. 21).

Bernal (1951) asserts: "Any self-subsisting and dynamically stable entity transforming energy from any source or, as Haldane put it, 'any self-perpetuating pattern of chemical reactions,' might be called 'alive.'. . . In this sense a galaxy or a star is alive, or, on a more terrestrial scale, a flame. Passing to a degree of complication greater than the biological, we might talk of the life of a human culture or civilization. All are characterized by birth, persistence and death . . ." (p. 14).

The second question, "What is an organism?", is generally answered by a definition. For example, Hopkins *et al.* (1952) state that "an organism may be defined as the smallest subdivision of a specific type of living matter which can carry on independently all activities necessary for the indefinite continuation of its life" (p. 3).

Whyte (1949) recognizes four classes of organisms: general, primitive, plant, and animal, and gives formulae for each. He asserts, "the General Formula for Organism: *An organism is a continuous normalizing process in a field of hereditary units*" (p. 150, italics his). By normalizing process, he means factors in the system which tend to restore "the normal resultant field after the relaxative process caused by any disturbance, and so maintaining the operation of cyclic components" (p. 105).

Sommerhoff (1950), after asking what life is and pointing out the need for a critical analysis of the problem, states, "A living organism may be described as a compact physical system of mechanically connected parts whose states and activities are related by an integrated set of directive correlations which, over and above any proximate focal conditions, have the continued existence of the system as an ultimate focal condition" (p. 195). Sommerhoff's assertion, when stripped of the qualifying language, is that a living organism is a physical system with a purpose. This definition is fundamentally the same as that of Whyte.

Bertalanffy (1952) ventures as a tentative definition: "A living organism is a hierarchical order of open systems which maintains itself in the exchange of components by virtue of its system conditions.

"This definition is certainly not exhaustive. It neglects a third attribute essential to living systems, namely, their historical character (pp. 109 ff.). With this reservation, however, it corresponds to the demands necessary in a scientific definition" (p. 129).

Each of these proposed definitions contains the assumption that the organism is a going, operating system of chemical reactions. But what these reactions are is not stated. Dixon (1948), in summarizing his lectures on multi-enzyme systems, is somewhat more specific regarding the nature of these reactions; however, he does not indicate precisely that he is considering an organism when he states, "For life to exist, we must have a certain minimum number of enzymes linked together in a system which can both bring about energy-yielding reactions and also transfer and use this energy for other processes" (p. 100).

The question, "How did life originate?", is generally answered more readily and with more agreement by writers. The hypothesis most frequently advanced is that before life originated, organic substances were developed by simple chemical or photochemical reactions. These substances reacted with themselves and produced more complex compounds. With the passage of time, the quantity and variety of organic substances increased. Finally, in this soup, particles formed, and somehow from them life originated. Haldane (1933), Oparin (1938), Holmes (1948), Lwoff (1951), Bernal (1951), Blum (1951) and Urey (1952) discuss aspects of this hypothesis.

Garrison *et al.* (1951) effected the formation of formic acid from carbon dioxide and water by radioactive techniques.

Regarding the origin of life, Osborn (1917) proposes that life originated with the development of a new form of material attraction in the ten chemical elements common to living organisms. This proposal contains the seeds of Schrödinger's hypothesis.

Horowitz (1945), as well as Troland, and Alexander and Bridges (Alexander, 1948), indicates that life originated with the formation of enzymes which satisfied their two criteria.

Blum (1951) suggests that if life arose in a complex mixture of organic compounds, some means of transporting free energy such as the adenylic acid-ADP-ATP system probably was the key factor in the transition from the non-living to the living state, and points out that although how this happened is not clear, the formation of a protein molecule seems more probable with the ATP-ADP system present than without.

The foregoing summary indicates that writers agree that life is a complex, apparently undefinable phenomenon, and that they generally assert that life appeared suddenly as a simple, poorly characterized organism in an organic milieu during Archeozoic time. These two statements lead to apparently paradoxical conclusions. First, if life is so complex that it can not be easily defined, then it seems absurd to suggest that it originated as a simple organism. Also, if life appeared as a simple organism, then the characteristics that differentiated that organism from its environment were elementary. Any hypothesis explaining what life is and how it originated must reconcile this apparent dilemma.

Discussion

What is life?

Each proposed solution regarding the origin of life depends ultimately upon its author's answer to the question, "What is life?". Therefore, a clear concept of what is meant by the term *life* is essential to theoretical biology. At present, the assumption underlying thinking on this subject seems to be that life is a universal, invariant and eternal something, of which each organism is a partial, imperfect and temporary representation. The precise nature of this something is not stated at present because (1) in the field of biology the need for such a definition has not been demonstrated; (2) biologic knowledge is too incomplete to warrant formulation of a definition; or (3) only teleological, purposive or cyclic definitions have been constructed. Although these reasons appear valid and compelling, they will vanish with the development of a definition of life which can be used in biological research and theory.

Because the current assumption is not amenable to simple and accurate statement and has not been expanded into a body of theory which unifies biology, a new postulate is proposed. *Life, regardless of what else it may be, is the manifestations of an organism.*

This assumption seems to be unsatisfactory because it implies that life is as varied as the myriads of individual organisms, dead or alive. However, the implication is of fundamental importance and actually strengthens the postulate. Organisms have many biochemical systems that are alike (Florkin, 1949; Umbreit, 1951); however, life in the form of a cow cannot be considered the same as life in the form of the grass she eats, when their common ancestral organism separated perhaps 1,000 million years ago; and life in the form of an amoeba is not identical with life in the form of man, when the parent of their ancestors divided say 750 million years ago.

If biology is a science, then biologists must assume that phenomena are manifestations of material systems, that generalizations or principles can be induced about

the manifestations and that nothing about the extra-phenomenal existence of these generalizations can be inferred. Organisms are, by definition, related if they are derived from identical ancestral forms. They, as material systems, change with environmental and genetic transformations; therefore, organisms separated from a common ancestor by but a brief interval have changed and are presumably different. Hence, manifestations of these organisms are different. If life is the manifestations of an organism, then the lives of individual organisms are different; if, however, life is a general principle with extra-protoplasmic existence, then nothing can be asserted about the life of organisms.

What is an organism?

The tentative assumption that life is the manifestations of an organism also appears to fail because the terms *manifestations* and *organism* have no valid meaning at present. *Manifestation* can be defined as anything perceivable by an observer. *Organism can be defined as a group of chemical systems in which free energy is released as part of the reactions of one or more of the systems, and in which some of this free energy is used in the reactions of one or more of the remaining systems.* The term "chemical system" is taken to include all of the elements and compounds necessary to a particular reaction. This definition of an organism is more general than the one suggested by Dixon (1948) and includes his as a special case.

Therefore, the complete new postulate is: life is anything perceivable by an observer of a group of chemical systems in which free energy is released as part of the reactions of one or more of the systems, and in which some of this free energy is used in the reactions of one or more of the remaining systems.

Coryell (1940) proposed the term *exergonic* for free energy-yielding, or $-\Delta F$ changes, and *endergonic* for free energy-using, or $+\Delta F$ changes. These terms will be used as synonyms.

The phrase, "anything perceivable by an observer," means, in part at least, the characteristics of life, because these are in reality generalizations about the observable phenomena pertaining to organisms. Hence, it seems probable that any definition of life must imply the existence of more than one organism. Whether or not the second organism, the observer, actually lives, is immaterial.

The phrase defined above as equivalent to an organism attempts to set this fundamental unit apart from the rest of the universe. Every organism known is a group of chemical systems of varying complexity and has some reaction or set of reactions which releases energy, and other reactions which use part of this energy; hence, modern forms satisfy the definition.

However, this definition of an organism raises two closely related questions: 1) When does the group of chemical systems become alive, and 2) what are the limits of the definition of *organism?*

The problem of when the group of chemical systems becomes alive has three possible solutions. First, the reaction which releases energy initiates life. This solution is unsatisfactory because many exergonic reactions which do not exhibit the characteristics of life are known. Second, the reaction using the energy begins life. This fails to give a solution also because many non-living endergonic reactions are known. Third, neither the energy-yielding reaction nor the energy-accepting one is alive; but together they form a system which manifests the characteristic phenomena of life and is interpreted to be alive. This solution requires that the definition of an organism be satisfied and at the same time introduces the notion that the uniqueness of an organism is based, in part, on the interdependent reactions of its component systems.

The question of the limits of the definition resolves into problems concerning

the upper and lower limits of organisms. The upper limit is not bounded. There is no way, at present, to predict the changes occurring in the descendents of existing organisms; organisms, in all probability, have but begun to explore the potentialities of the systems of energy-releasing-energy-using reactions. The lower limit is bounded. Superficially, this boundary appears to be an autotrophic group of enzymes (at present unknown); however, the definition does not require that the simplest organism have any organic molecules. The only conditions are that an energy-releasing system and an energy-using system form a group. Therefore, any pair of chemical reactions satisfying these conditions is an organism. Things as simple as two inorganic reactions meet the definition. This conclusion agrees with the suggestion of van Niel (1940), that any chemical process which liberates free energy can be used by some living organism as the main, or sole means of fulfilling its energetic requirements.

Consideration of the manifestations of an inorganic organism discloses something of the nature of primitive life. This organism has, by definition, at least one energy-yielding reaction (catabolism) and also one or more reactions which use energy in the synthesis of new compounds (anabolism). The nature of these groups of reactants determines the composition of the organism and its form. The duration of the set of reactions as an organism depends primarily upon the arrangement of the various interacting substances, their physical states and the continued maintenance of the system. Precisely how this could occur in inorganic reactions is difficult to see; however, some reaction in which one of the end products was colloidal might provide a partial answer. If the end products of the reactions accumulate, the organism increases in volume; but if these substances volatilize or precipitate, leaving its proximity, no increase occurs. If the concentration of any of the reacting compounds changes, then the rate of that reaction and of all dependent ones changes. Hence, the organism responds to chemical stimuli. Very likely the organism sometimes separates into two or more parts by the mechanical action of currents in the milieu. If each of the parts contains some of each of the reacting chemical systems, and if they drift into supplies of raw materals, then these organisms can continue their reactions and enlarge.

Briefly, the inorganic organism is characterized by a typical composition, metabolism and the ability to respond to chemical stimuli; it probably also exhibits growth, internal molecular movements and perhaps occasionally reproduces by accident; and it presumably does not move independently. Thus, this simple organism exhibits most of the phenomena of life and therefore is interpreted as being alive, although it does not possess either of the two "essential criteria of life" of Troland and of Alexander and Bridges (Alexander, 1948). Therefore, it is more primitive than the ones postulated by these workers.

How did life originate?

The third question, "How did life originate?", immediately raises problems regarding the environmental conditions and the types of energy-releasing and energy-using reactions existing at the time of origin.

Environmental conditions: Statements concerning the environment must be highly speculative because of the vast temporal distance separating us from the first organism. Knopf (1949) states, "summarizing the age evidence based on radioactive minerals, we find the oldest minerals are 2,000 million years old. At least three-fourths of geologic time, 1,500 million years, had passed before the beginning of Cambrian time, when life other than algae first became abundant on this planet, or at least left records in the rocks" (p. 6). Marble (1950) summarizes the evidence and concludes, "Age

of crust of earth [is] . . . uncertain, but the order of magnitude is about 3,250 million years" (p. 18). Blum (1951), Bernal (1951) and Urey (1952) discuss the probable conditions existing on the primitive earth at the time life appeared; therefore, they will not be considered here. Most workers assume that, except for a possible increase in the amount of oxygen in the atmosphere and a probable decrease in radioactivity, the physical and chemical conditions have not changed noticeably since the earth originated. They further suggest that by various means a large number of organic compounds were synthesized before the coming of life. Hence, according to rules now in force, a writer on the origin of life has available any substances which his theory requires. The one thing known absolutely about that ancient world is that until the first organism originated the world was bacteriologically sterile.

Exergonic reactions: The main types of reactions which might supply energy for an organism are four: radioactivity, inorganic oxidations or dehydrogenations, photolysis and organic oxidations, either aerobic or anaerobic. Garrison *et al.* (1951) demonstrated that a radioactive energy source can reduce carbon dioxide to synthesize formic acid. This is an organism according to the definition and will be considered later. Several groups of bacteria, the chemosynthetic organisms, oxidize inorganic compounds, such as hydrogen sulfide, thiosulfate, sulfur, ferrous compounds, ammonia or nitrites with release of energy. Other kinds of bacteria, as well as the phytoflagellates, the algae and the green plants, use light energy to dehydrogenate water or some other hydrogen donor and reduce carbon dioxide to organic substances. Many organisms oxidize organic compounds to release energy. Some convert the entire compound to carbon dioxide and water by means of reactions ultimately involving molecular oxygen; others effect a partial conversion of the compound to intermediate substances in the absence of oxygen. Some of the energy released by these reactions is used in other reactions to reduce inorganic and organic compounds, especially carbon dioxide, to synthesize new and more complex compounds and to initiate reactions between components of these systems. The results of these energetic reactions appear to the observer as manifestations of a living organism.

The question immediately arises as to which of the energy-releasing-energy-using systems actually occurred and which one was ancestral to modern organisms. Although Garrison *et al.* (1951) showed that radioactive material can supply energy to reduce carbon dioxide to formic acid, the natural occurrence of this system as a living form seems highly improbable, because the supply of energy, that is, radioactive decay of an element, is variable, and because as the organic molecules increase in number, the probability of high energy particles striking and disrupting some of them increases.

If radioactive systems do not supply energy for organisms, two other general methods of acquiring energy are available, namely, autotrophic and heterotrophic sources. Fundamentally, autotrophic systems obtain their energy from light or from the oxidation of inorganic compounds and use this energy to reduce carbon dioxide; whereas heterotrophic ones oxidize organic compounds, either anaerobically or aerobically, to supply energy. Many modern organisms are known which combine some of the nutritional properties of autotrophs and heterotrophs. For this reason Werkman (1951) believes that the distinction between these exergonic patterns is more apparent than real; however, for theoretical simplicity it is postulated that the primitive organisms considered in this study utilized only one type of nutritional behavior.

Hypotheses concerning the origin of life. a. *Heterotrophic:* Osborn (1917) assumed that the first organisms were

autotrophs. Most other students (Oparin, 1938; Horowitz, 1945; Dauvillier, 1947; Alexander, 1948; van Niel, 1949; Bernal, 1951; Blum, 1951; Lwoff, 1951 and Urey, 1952) prefer a combined photosynthetic-heterotrophic origin in general similar to Haldane's hypothesis (1933):

> Now, when ultraviolet light acts on a mixture of water, carbon dioxide, and ammonia, a vast variety of organic substances are made, including sugars, and apparently some of the materials from which proteins are built up. This fact has been demonstrated in the laboratory of Baly of Liverpool and his colleagues. . . . But, before the origin of life they (organic substances) must have accumulated till the primitive oceans reached the consistency of hot dilute soup. Today an organism must trust to luck, skill, or strength to obtain its food. The first precursors of life found food available in considerable quantities, and had no competition in the struggle for existence. As the primitive atmosphere contained little or no oxygen, they must have obtained the energy which they needed for growth by some other process than oxidation—in fact by fermentation . . . (p. 149).

In this outline of the photosynthetic-heterotrophic origin of life are many of the points of all subsequent theories that begin with the "hot dilute soup," including Oparin (1938), Horowitz (1945), Bernal (1951) and Urey (1952). Haldane's hypothesis is based on the "demonstrated fact" of Baly and his colleagues. Recent work in photosynthesis had suggested that a more complex system is necessary. Van Niel (1949) states, "However, numerous reinvestigations of this phenomenon by various competent workers have so completely failed to corroborate Baly's results that it seems entirely legitimate to disregard this work here" (p. 439).

Van Niel's statements effectively remove the basis of Haldane's phototrophic-heterotrophic hypothesis; however, Bernal (1951) and Urey (1952) propose independently that photochemical processes using ultraviolet light or electrical phenomena occurred in the upper layers of a reducing atmosphere which resulted ultimately in the synthesis of organic compounds. These accumulated in the lifeless primitive seas and reacted with each other to produce many different substances including complicated ring systems such as porphyrins. Eventually in this soup life arose. These speculations which are based on the assumption that the oxygen of the early atmosphere had been depleted entirely by oxidation of reduced elements are to be questioned seriously because few, if any, chemical reactions are known which are so complete that one of the reactants is totally exhausted. If some free oxygen existed, the atmosphere was not reducing. Then, the proposals of Bernal and Urey are not valid, and no source of organic matter is available for the heterotrophic origin of life. Blum (1951), after reviewing the various mechanisms proposed to explain the origin of atmospheric oxygen, concludes:

> It seems reasonable to think, however, that for as long as there has been an atmosphere it has contained some O_2, and that there has never been a strictly anaerobic period, although the concentration of this component may once have been quite low. The manner of the origin of life has often been associated with ideas about the presence or absence of O_2. . . . In the present state of our knowledge it seems rather dangerous to tie our ideas of the origin of life too definitely to one or the other contingency (pp. 51–52).

In a discussion of the origin of life Urey (1952) suggests that the photochemical processes continued until the ancient oceans whose volumes he estimates at ten per cent of present seas were approximately ten per cent solutions of organic matter and that this would provide a favorable environment for the origin of a living organism. If the estimate of Daly (1951) that the volume of the modern oceans is 1.49×10^9 km.^{3}, then about 1.5×10^7 km.^{3} of organic material was produced photochemically. If the sterile era of the earth was about 1.5×10^9 years, the average rate of formation of these substances was 10^{-2} km.^{3} per year. This, assuming a density of one, a relatively low value, is approximately 10^7 metric tons per year. Any hypothesis

which requires continuous, non-living, organic syntheses on this scale overwhelms the imagination and stretches credulity to the breaking point. Thus, the probability of the occurrence of a heterotrophic origin seems to be slight.

A further argument for the heterotrophic origin of an organism has been adduced by Horowitz (1945), who proposes:

> . . . that the first living entity [a self-duplicating nucleoprotein] was a completely heterotrophic unit, reproducing itself at the expense of prefabricated organic molecules in the environment. A depletion of the environment resulted until a point was reached where the supply of specific substrates limited further multiplication. By a process of mutation a means was eventually discovered for utilizing other available substances. With this event the evolution of biosyntheses began. The conditions necessary for the operation of the mechanism ceased to exist with the ultimate destruction of the organic environment. Further evolution was probably based on the chance combination of genes resulting to a large extent in the development of short reaction chains utilizing substances whose syntheses had previously been acquired (p. 156–157).

The hypothesis advanced by Horowitz assumes first, that life is embodied in a "self-duplicating nucleoprotein," i.e., the Troland and Bridges and Alexander hypothesis; second, that independent, chance reactions account for the syntheses of all the organic compounds which occur in the original nucleoproteins; third, that these organic substances were developed at random in the ancient seas in quantities sufficient to produce at least local concentrations which reacted to form a molecule of nucleoprotein; and, finally, that this initial nucleoprotein was the specific gene capable of originating the enzyme needed for the first step in the oxidation of an unnamed organic compound. The probability that the "first living entity" satisfied all of these assumptions seems infinitesimal, especially when no source of energy is proposed to aid in the synthesis of the component parts of the nucleoprotein molecule. This necessitates the examination of Osborn's autotrophic hypothesis.

b. *Autotrophic:* Osborn speculates that the first organism originated as a new form of unity in the cosmos by the mutual action and reaction of a group of life elements. The grouping of these elements occurred in a colloidal state. The organisms soon evolved various "chemical messengers," such as enzymes and hormones. In this assemblage "there arose the rudiments of competition and Natural Selection which tested all actions, reactions and interactions of two competing individuals."

These speculations ignore such fundamental problems as the source of energy for the new organisms and the method of reproduction. However, Osborn considers the source of energy when he discusses the "biochemical evolution of the bacteria." He says, "In the origin of life bacteria appear to lie half-way between our hypothetical chemical precellular stages and the chemistry and definite cell structure of the lowliest plants, or algae" (p. 80). Further, "In their power of finding energy or food in a lifeless world the bacteria known as *prototrophic,* or 'primitive feeders,' are not only the simplest organisms, but it is probable that they represent the survival of a primodial stage of life chemistry. These bacteria derive their energy and their nutrition directly from inorganic chemical compounds: such types were thus capable of living and flourishing on the lifeless earth even before the advent of continuous sunshine and long before the first chlorophyllic stage (Algae) of the evolution of plant life" (pp. 81–82). As "primitive feeders" he recognizes ammonia and nitrite oxidizing forms, as well as iron and hydrogen sulfide oxidizers.

In this autotrophic theory, Osborn has developed in broad outline a probable sequence of events in the origin of life. A number of omissions and weaknesses appear on close inspection; however, he introduces a source of energy for his "hypothetical chemical precellular stages"

soon after their assumed origin. This energy source, the oxidation of ammonia to nitrite, is still a real phenomenon in the metabolism of some bacteria. Van Niel (1949), Lwoff (1951), and Bernal (1951) assert that the earliest organisms were nonphotosynthetic; however, they do not furnish details regarding these autotrophic forms; and they do not indicate their role except as sources of organic compounds in the evolution of photosynthetic organisms.

Umbreit (1947) and Snell (1951) argue against the autotrophic theory of origin and for the heterotrophic one, asserting that: "Simplicity in internal enzymatic constitution, not complexity, would be expected in the first living organism. Such simplicity would require a complex organic nutrition" (Snell, p. 222). This argument is based on the assumption that modern autotrophic metabolism is essentially the same as heterotrophic nutrition with the added condition of mechanisms for the autotrophic acquisition of energy. The validity of this assumption even in recent organisms is subject to doubt (Baalsrud and Baalsrud, 1952); and the implication that ancient autotrophs had the same nutritive systems as living forms is certainly unwarranted.

Steps changing first organisms into modern forms

The question, "How did life originate?", implies the corollary, "What were the steps which occurred to change the first organisms into modern biologic forms?" Frequently, the corollary is meant when questions regarding the origin are asked. The reason for this is that for any hypothesis about the first organisms to be useful, the primitive form must give rise to modern ones by means of a series of changes which modify the simpler organisms and which are passed by them to their descendents.

Only the changes that lead to the development of a few fundamental characters can be considered and these must be presented in broad outline. Attempts at detail would imply the existence of a body of knowledge which is at present lacking.

It seems probable that from the beginning of the earth chemical reactions occurred. Some of these were oxidations or dehydrogenations, and released energy as one of the end products. Usually, the energy was dissipated; however, occasionally some of it was captured by components of an energy-using system. This group of chemical systems satisfies the definition of an organism, and the phenomena manifested by them is, by definition, life.

Presumably, this occurred many times, in many places. However, probably most of these simple forms became extinct when supplies of raw materials were exhausted, or when end products accumulated. Some survived. These, very likely, were organisms which used common substances such as hydrogen sulfide or ammonia for energy.

The assertion can be made that most of these ancient inorganic organisms were not alive because they existed but briefly and then ceased to be. This objection is based on the assumption that all organisms have a degree of permanence similar to that found in present day species, a continuous life spanning many generations. Evidence for this assumption is lacking. Continuity is not generally considered as a characteristic of life, although Bernal (1951) suggests that such is the case.

Once an inorganic organism forms, it can remain static continuing the same reactions, or it can change into a different form. The primitive organism can evolve in one or more of the following ways: The energy-producing and/or the energy-using reactions can change, new methods of acquiring and/or using energy can develop or the technique of transferring energy from one reaction to another can change. If one or more changes occur, and if they persist, the

subsequent behavior of the organism can be affected significantly.

The exergonic part of a primitive organism presumably was initially slow and erratic, producing similar behavior in the energy-using reactions. If, by diffusion, some inorganic substance capable of catalysing the energy-producing reaction entered the system, and if the catalytic activity caused an increase in reaction rate, then more energy would be released per unit time. This very likely would produce an increased use of energy, resulting in an acceleration in the apparent behavior of the organism.

At present, almost nothing is known about the methods of acquiring distinctly new sources of energy, hence, statements on this subject are completely speculative. Early organisms presumably oxidized many inorganic substances with the release of energy. If the elements of an energy-producing reaction entered the neighborhood of a primitive organism, probably at times the organism adopted the new source of energy. In the long run, compounds of but four elements—sulfur, nitrogen, iron and molecular hydrogen—had survival value, and organisms which obtained energy by oxidizing one or more of these substances are ancestral to modern forms.

The endergonic part of a simple organism, very likely irregular and slow because of the difficulty in acquiring free energy, would improve if some inorganic substance capable of catalytic activity entered the neighborhood of the reaction. If the catalyst increased the rate of reaction, the visible activity of the organism increased also.

Although many inorganic reactions which could be energy-acceptors are available, the ones which actually used energy in the primitive organism are unknown. However, not any of the numerous organic energy-accepting systems used by modern organisms were available to the earliest forms. At this stage, the organism presumably acquired new uses for free energy by the diffusion of the components of acceptor reactions into its vicinity. The importance of these inorganic systems in the economy of the early forms can not be estimated. Among the acquired energy-consuming reactions, presumably, was a method of reducing carbon dioxide to an organic compound. Werkman (1951) asserts that carbon dioxide assimilation and subsequent carboxylation of acetate occurs at a high redox potential (0.22 volt), and Garrison *et al.* (1951) indicate that iron sulfate catalyzes the carbon dioxide to formic acid reaction when radioactive materials supply energy for the reaction. This reaction is fundamental in the development of present biological form, and the organism which acquired it simultaneously gained a potential advantage over its contemporaries.

All known organisms have organic phosphate compounds which store the free energy released by the exergonic reactions and later liberate it to energy-using systems. Whether inorganic systems with similar functions developed in the first organisms is unknown; however, because the phospho-organic compounds occur in all organisms, they presumably arose early in the evolution of biological systems. Blum (1951) emphasizes the importance of the origin of the adenylic acid system, although he places it earlier in the evolution of organisms than is hypothesized here, when he suggests that a "means of free energy transport may have been this key factor in the transition from the non-living to the living state" (p. 168).

The synthesis of these substances which seem to characterize the next state in the history of living forms must be considered.

If an inorganic organism evolved so that its exergonic reaction oxidized hydrogen sulfide, and its endergonic one reduced carbon dioxide to an organic compound, such as formic acid, and if these reactions were accelerated by the catalytic activities of inorganic substances, the resulting organism could po-

tentially produce adenylic acid. Baalsrud and Baalsrud (1952) consider that in the thiobacilli the assimilation of carbon dioxide is closely and intimately connected with the oxidation of sodium thiosulfate. This is contrary to the findings reported by Vogler and Umbreit (1941), Umbreit, Vogel and Vogler (1942), Vogler, LePage and Umbreit (1942), Vogler (1942 a and b), Vogler and Umbreit (1942), LePage (1942), LePage and Umbreit (1943 a and b) and Umbreit (1951) that the adenosine phosphate system provides an energy transport link between the oxidation of sulfur and the reduction of carbon dioxide in *Thiobacillus thiooxidans*. However, it provides for biological theory a simpler mechanism for the reduction of carbon dioxide than was previously available.

The form of this organism can only be speculated. Osborn (1917) believes that the original living forms were colloidal, and Oparin (1938) suggests that they were proteins which had separated from solution. Haldane (1933) proposes that they were oily films on the surface of ancient seas. Bernal (1951) presents arguments to show that the first forms were adsorbed on particles of clay; whereas Pirie (1951), in discussing Bernal's hypothesis, prefers "V. M. Goldschmidt's ideas of absorption of minerals from which an active element can be eluted with difficulty" (p. 70).

Actually, any or all of these proposals, except for that of Oparin, could serve. If the catalytic materials were colloidal particles, the suggestions of Osborn, Bernal or Goldschmidt could be correct; however, if the catalysts were ions or molecules of inorganic matter, Haldane's idea might be valid.

Regardless of its morphology, this organism would require definite compounds for its exergonic and endergonic reactions. Unless hydrogen sulfide, oxygen, carbon dioxide and water, as well as some inorganic salts and possibly ammonia, were present in the environment, the postulated energy-yielding and energy-using reactions would not occur. All of these substances presumably were present in the early seas.

If the organism obtained the needed raw materials from the environment and if the assumed reactions occurred, then sulfur and very likely formaldehyde or formic acid would form in its neighborhood. The sulfur would probably precipitate, and molecules of the organic compound would accumulate near the site of formation, although some of them would diffuse away.

As the concentration of organic molecules increased, the opportunity for reactions to occur between them also increased. Probably some of the energy released in the oxidation of hydrogen sulfide actuated these reactions. Very likely, the result was the formation of many simple carbon compounds including pentose and hexose sugars. Some of these substances presumably reacted with ammonia to form amino acids and pyrimidine bases such as uracil, thymine and cytosine. One of these latter compounds reacted with ammonia and a methyl compound to form the purine base, adenine. If a molecule of adenine and one of the pentose, d-ribose, united with the loss of a molecule of water, a molecule of adenosine would result. If this compound combined with a molecule of phosphoric acid, adenylic acid (adenosine monophosphate) would result.

Although the original synthesis of adenylic acid in all probability did not follow the proposed sequence of events, the suggested outline contains a novel element not found in other theories; namely, that the oxidation of an inorganic compound provided free energy for the endergonic reactions necessary for effecting the formation of a moderately complex organic molecule.

That the synthesis of adenylic acid proceeded in an orthogenetic manner is unlikely; instead the production of the necessary intermediates probably went by fits and starts with many side reactions producing other organic substances.

Therefore, with the attainment of a molecule of adenosine monophosphate many compounds of carbon also became available to the primitive organism. In fact, the neighborhood of the organism for the first time became predominantly organic.

With the advent of adenylic acid the metabolism of the organism probably changed markedly. Adenosine monophosphate (AMP) can combine with phosphoric acid provided sufficient free energy is available to form adenosine diphosphate (ADP). With more free energy this compound can unite with another phosphoric acid molecule to produce adenosine triphosphate (ATP). The formation of the di- and triphosphates of adenylic acid is attained with difficulty in the biochemical laboratory. However, these substances are essential in the metabolism of all organisms; hence, their synthesis must be assumed early in the evolution of organisms although the mechanisms involved are almost entirely unknown. These phosphates of adenylic acid are energy storage substances, and they release free energy and phosphoric acid readily to other compounds by reversing the AMP to ADP to ATP reactions. By means of this system some of the free energy obtained by the oxidation of hydrogen sulfide can be stored and released at a later time and in another place. This effects endergonic reactions at a distance from the site of oxidation and introduces the mechanism of coupled reactions into the organism.

Once an organism acquired the adenosine energy transport system, this became the primary energy acceptor of the exergonic reaction and the main source of energy for the endergonic reactions. This organism would appear to be, according to modern biochemical thinking, a set of primitive energetically coupled reactions with adenosine phosphate compounds functioning chemically as energy mediators (Van Niel, 1940; Lipmann, 1941; Kalckar, 1941). Very likely the organism with this technique achieved an efficiency markedly greater than that of its neighbors and with this a significant potentiality for survival. Further, it was the probable ancestor of all subsequent organic organisms.

Almost all authors (Osborn, 1917; Troland, and Alexander and Bridges [Alexander, 1948]; Haldane, 1933; Oparin, 1938; Horowitz, 1945; Dauvillier, 1947; Bernal, 1951 and Urey, 1952) consider that life first appeared as a protein molecule capable of self-duplication, a nucleoprotein. This molecule is presumed to have originated by chance from a mixture of amino acids. Blum (1951) points out that the chance formation of "tripeptides would be about one-hundredth that of forming dipeptides, and the probability of forming a polypeptide of only ten amino acid units would be something like 10^{-20}" (p. 163). He suggests, however, that some exergonic reaction either organic or inorganic could supply energy and that the adenosine system could transfer some of this energy to effect the formation of peptide linkages between molecules of amino acids. He further hypothesizes, "If the adenylic acid system introduced the purine ring pattern which latter became incorporated into nucleic acid structure, it seems reasonable to suppose that this pattern was perpetuated in association with nucleoproteins" (p. 169). Earlier, Kalckar (1941) suggested that the energy required for the formation of the peptide bond is furnished by the adenosine triphosphate to diphosphate reaction. Cohen and McGilvery (1946), and Borsook and Dubnoff (1947) demonstrated that the synthesis of *p*-amino hippuric acid from glycine and *p*-amino benzoic acid and of hippuric acid from glycine and benzoic acid, respectively, in the liver, are dependent on the presence of adenosine triphosphate. Friedberg, Winnick and Greenberg (1947) established that glycine is incorporated into tissue proteins only in the presence of adenosine triphosphate. Hence, Blum's proposal for the mechanism of the syn-

thesis of protein to form the first organism is a biochemical fact in rats.

In his discussion of the type of metabolism of the first organism, Blum (1951) raises a more fundamental problem when he poses the riddle: *"How, when no life existed, did substances come into being which today are absolutely essential to living systems yet which can only be formed by those systems?* It seems begging the question to suggest that the first protein molecules were formed by some more primitive 'nonprotein living system,' for it still remains to define and account for the origin of that system" (p. 170, italics his). Because the inorganic organism is defined and because the events necessary for its origin have been outlined, the following tentative solution to his riddle, based on the activities of this organism, is hypothesized: During the steps leading to the synthesis of adenylic acid both pyrimidine and purine bases were formed. If these united with molecules of pentose sugar and of phosphoric acid, a nucleotide would be formed. The adenosine triphosphate to adenosine diphosphate reaction could supply the required energy and the phosphoric acid. If the sugar was d-ribose, ribonucleotide was produced; if, however, it was desoxyribose, desoxyribonucleotide was synthesized. Both occur in modern organisms and were probably formed. By condensation reactions polynucleotides could be synthesized. Not any of these substances have been produced in the laboratory; hence, all of the details are speculative. Brown, Roll and Weinfeld (1952) and Buchanan (1952), in discussions of the biosynthesis of nucleic acids and their precursors, indicate that the mechanisms are poorly understood but organisms frequently use several paths to produce the same compound.

If amino acids were produced in the secondary reactions between the first organic compounds and ammonia, then these could react as Blum (1951) proposes, to form polypeptides or even proteins with the needed energy supplied by the adenylic acid transfer system. If a protein combined with one or more molecules of polydesoxyribonucleotide, a nucleoprotein would result. This compound could satisfy the requirements for the original living "organism" proposed by most writers, namely, self-duplication and enzymatic action. The expression of these properties would depend primarily on the presence of numerous compounds in the vicinity of the organism. How the nucleoproteins could express these activities is not known at present. However, these mechanisms are not understood in the nuclei of modern organisms in which similar complex genetical syntheses are routine.

If the nucleoprotein satisfied these requirements, then the organism would have a one-gene-one-enzyme system which would fit the hypothesis of Beadle (1945). Then, if the required starting substances were available in the environment, the nucleoprotein could reproduce itself and also produce molecules of enzyme. This is an almost completely organic organism capable of reproducing part of itself by genetic methods.

The organism with its self-reproducing enzymes capable of oxidizing hydrogen sulfide would produce large amounts of free energy. With the increase in available energy the synthetic activity of the organism would accelerate resulting in the stepped up production of organic compounds. This would effect in part at least an increase in the amounts of adenylic acid and nucleoprotein present. These compounds would abet the chances of survival for portions of the organism which became separated from the main body. Thus, a simple mechanism for genetic continuity of the organism and its progeny developed with the formation of the first nucleoprotein molecule, and Blum's riddle is tentatively answered.

In a mixture such as the primitive organism, the chances for errors in the duplication of molecules of nucleoprotein would be large. Hence, compounds dif-

fering in structure from the parent proteins, if they had enzymatic activity, would influence the metabolic processes of the organism. Genetically, they would be mutations. Progeny inheriting these mutants would differ from the parent organism and would respond differently to environmental factors. Thus, chance variations in the formation of nucleoproteins caused changes in genetic structure (mutations); and when these variations were transmitted to offspring, genetic evolution occurred.

At this stage, the hypothetical organism would consist of the hydrogen sulfide oxidizing and the carbon dioxide reducing reactions, several nucleoproteins capable of synthesizing the enzymes necessary for catalyzing the exergonic and endergonic systems, an adenylic acid energy transfer mechanism and numerous organic intermediates produced as secondary reactions. In size the organism would still be submicroscopic with diffuse boundaries; however, it would exhibit all of the characteristics of life. Were its discovery announced and verified, it would be accepted as a living organism and would be classified either as the presumed ancestor of the sulfur oxidizing organisms or as a degenerate offshoot of them occupying a systematic position somewhat analogous to that of the viruses.

In this organism are the beginnings of the mechanisms needed for the evolution of all subsequent living forms. Some of the stages in this evolution are the formation of a limiting membrane which surrounded the organism and partially separated it from the environment, the clumping together of the genetic substances into a distinct compact mass separated from the biochemical activity inherent in the energy-producing and energy-using systems, and the development of new exergonic and endergonic reactions. These latter developments depend upon the formation of new enzymes by mutation.

Three types of new energy-yielding systems can develop. The organism might acquire the techniques for oxidizing new and different inorganic compounds. If photo-accepting compounds developed, then the organism could use the radiant energy in place of that obtained by oxidation of inorganic substances in the reduction of carbon dioxide to organic matter. This organism would have, in general, a marked advantage over the chemosynthetic ones; and photosynthetic forms presumably would soon dominate those areas of the environment which were illuminated. With the activity of the autotrophic organisms, organic matter would accumulate in the environment. Organisms which lost their chemosynthetic enzymes or photosynthetic pigments might survive if they had acquired enzymes capable of oxidizing some of the organic compounds which diffused into them. In the evolution of heterotrophic nutrition very likely those systems present in the organism would be used with minimal modifications and but a few specific enzymes would be required *de novo*.

The foregoing discussion is speculative, and very likely the precise sequence of events differs markedly from the one proposed. However, an attempt has been made to derive the organic world from the inorganic one, using only chemical and physical methods at present available and at the same time presenting the story as a consistent, evolutionary system. Those phases of the origin which other writers consider important have been placed in their apparently logical positions in the scheme. How valid the proposed hypotheses are can not be assessed immediately. No recipe for the manufacture of an organic form is given; however, many groups containing simple inorganic reactions can be constructed. These, by definition, are organisms, and their biological characteristics should be studied. Further, the biological behavior of systems containing the adenylic acid energy transfer mechanism should be in-

vestigated. As analytical biochemistry progresses, attempts to apply its findings in light of the results obtained with these simple organisms should be made.

Until chemical systems can be constructed and integrated so that organisms of specified degrees of complexity result, biology as a science must remain in many ways about equivalent to the alchemical stage of chemistry.

Summary

To summarize, the hypotheses advanced are based on a new assumption: life is the manifestations of an organism, where manifestation is defined as anything perceivable by an observer, and organism is defined as a group of chemical systems in which free energy is released as part of the reactions and in which some of this free energy is used in the reactions of one or more of the remaining systems. Any pair of chemical reactions satisfying these conditions is an organism. Things as simple as two inorganic systems can meet the requirements.

This assumption when applied to the problems of the origin of life and the evolution of early organisms seems to have advantages which all other proposals lack. First, no large quantities of organic substances synthesized by totally inorganic processes are required; also, a source of energy is made available to the organism for its synthetic activities from its earliest instant of existence.

Presumably, chemical reactions occurred from the beginning of the earth. If an oxidative system released free energy as an end product and if the components of an endergonic reaction absorbed some of this energy, an organism was formed, and life originated with the manifestations of its chemical activities. At first, the reactions very likely were slow and erratic; however, if some of the substances which diffused into the neighborhood of this organism had catalytic activity, the reactions would become surer and more stable.

In time, the component parts of new and presumably more efficient reactions diffused into the organism and became part of its metabolism. Finally, in this evolutionary development, the organism acquired as the exergonic reaction the oxidation of hydrogen sulfide to sulfur, and as the endergonic one the reduction of carbon dioxide to an organic compound such as formaldehyde or formic acid. With the passage of time molecules of the organic compound would tend to accumulate near the site of formation. As the concentration of organic molecules increased, the opportunity for reactions between them and compounds in the vicinity also increased. Probably some of the energy released by the exergonic system aided these reactions. Very likely the result was the formation of many simple carbon compounds, such as sugars and amino acids. Presumably, with time a purine base was formed which united with d-ribose and phosphoric acid to produce adenylic acid. With the origin of this molecule, the adenosine phosphate energy transfer system arose, and the organism had a method for storing energy temporarily which increased its metabolic efficiency markedly.

With the adenylic system supplying the energy, probably both nucleotides and proteins were synthesized from compounds formed as secondary reactions in the organism. If these compounds united, a nucleoprotein molecule, presumably capable of self-duplication, would result. If the protein had catalytic activity, then on the one-gene-one-enzyme hypothesis the organisms would presumably have both the ability to synthesize new molecules of nucleoproteins and enzymes. If the enzyme was capable of oxidizing hydrogen sulfide, large amounts of free energy would be available for endergonic reactions. This would increase the metabolic and synthetic activities of the system.

With the formation of the self-duplicating nucleoprotein, the organism acquired a crude method of genetic con-

tinuity and reproduction. Because in the simple organism the chances for errors in the duplication of molecules of nucleoprotein would be large, occasionally molecules differing from the parent substance would be formed. These new nucleoproteins, if they had enzymatic activity, would influence the metabolism of the organism and genetically would be mutations. With the chance variations in the formation of nucleoproteins, the possibility of genetic evolution arose.

At this stage, the organism, which would be submicroscopic in size with diffuse boundaries, would contain hydrogen sulfide oxidizing and carbon dioxide reducing reactions together with several genes and the enzymes synthesized by them, the adenylic acid energy transfer system and numerous organic intermediates produced by secondary reactions. It would exhibit all of the characteristics of life, and if it were discovered, it would be accepted as a living organism.

In this organism are the beginnings of the mechanisms required in the evolution of all subsequent living forms.

"The above conclusions are speculative. They will remain so until living creatures have been synthesized in the biochemical laboratory" (Haldane, 1933, p. 153).

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SELECTION BY PREDATION ON ALBINO AND NORMAL SPADEFOOT TOADS

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Observations of selection through predation in nature are rare. Experimentally, operation of natural selection was first definitely demonstrated by Sumner (1934, 1935), while Carrick (1936), Iseley (1938), and Dice (1947) have contributed much to the understanding of the role of selection in a predator-prey relationship. The entire problem of selection has been discussed recently by Allee *et al.* (1949). The discovery of a vernal pool containing a population of both albino and normal spadefoot toad larvae (*Scaphiopus h. hammondii* Baird) at the San Joaquin Experimental Range, O'Neals, Madera County, California, on April 11, 1952, permitted an observation of selection by predation under field conditions.

Acknowledgments are due S. F. Wood and R. F. Shaw for valuable suggestions and W. E. Howard, F. A. Pitelka, and R. C. Stebbins for critically reading the manuscript. The California Forest and Range Experiment Station, U. S. Forest Service, provided facilities for the study.

THE EXPERIMENTAL POPULATION

The population used in this experiment was found in a pool located in the "natural area" or ungrazed portion of the Experimental Range. It was a small pool, measuring six feet by eleven feet and was about one foot in greatest depth (fig. 1). In the bottom of the pool there was a growth of dark green, vascular, aquatic vegetation and the remnants of terrestrial grasses which grow each year after such pools dry up. The water was clear. The entire population of larvae was caught, taken to the laboratory, and counted. Some larvae of the Pacific tree toad (*Hyla regilla*) were found and removed. In the entire population there was little variation in total length (fig. 2), and hence the tadpoles were considered to be of more or less the same age. Of the population of 1,452 larvae, 298 or 20% were true albinos; that is, the eyes were pink.

A sample of 100 of each of the albino and normal forms, all approximately equal in size, was returned to the pool on April 12, 1952. A fence, eight inches in height, of 1/4-inch mesh hardware cloth, was constructed around the pool, one foot from the edge. This fence retained the young metamorphosed toads, but it did not deter predators such as birds and raccoons.

CONTROLS

To provide situations where differences in survival would not be due to predation, controls were set up. It proved impractical to use comparable pools. Two watering troughs not currently being used by cattle served for this purpose. In a half-cylindrical, metal trough (10′ × 3–1/2′ × 15″), 100 each of the two forms were placed. This trough, raised off the ground about two feet, was coated on the inside with a layer of tar upon which algae grew abundantly. The presence of a small creek ten yards away probably reduced the chance that predators would investigate it.

In a concrete trough, 46 albinos and 23 normals were placed. It was rectangular (24′ × 4′ × 18″), rested on the ground, and was divided in two equal sections, one-half containing a solid mat of *Anacharis* (= *Elodea*) floating on the surface, the other half containing a bottom-dwelling celery (*Vallisneria*). The tadpoles were released in the latter end

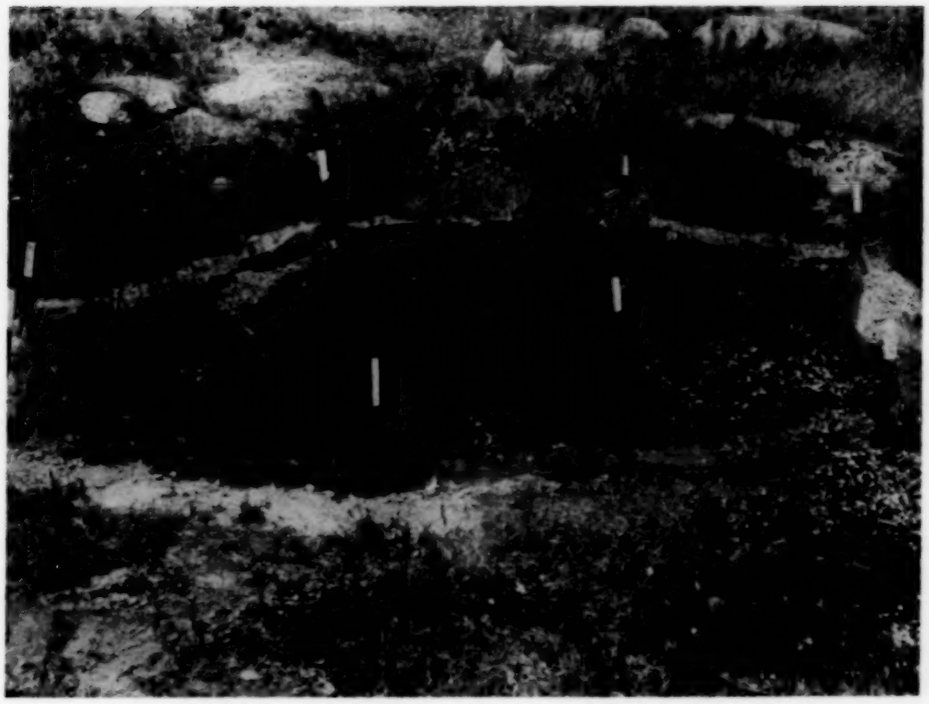

FIG. 1. The pool in which albino and normal spadefoot toads were found and where raccoon predation took place, showing the reduction in size at the end of the experiment. U. S. Forest Service Photo by Lisle Green.

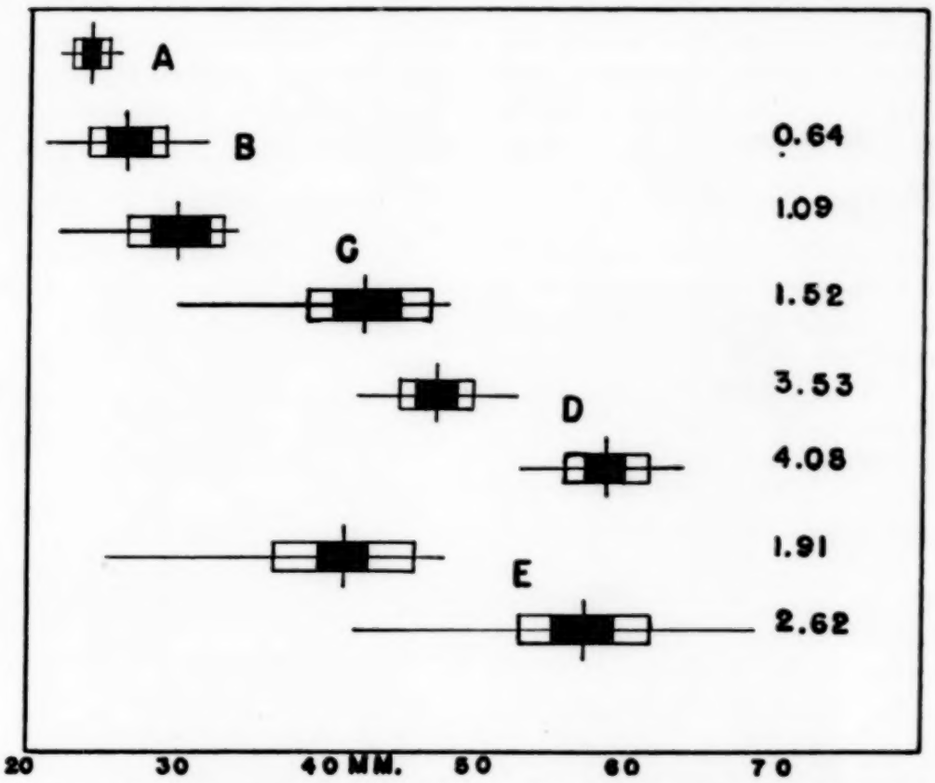

FIG. 2. Comparison of total lengths of albino and normal spadefoot toad larvae, using the method of Dice and Leraas (1936). Statistically significant differences exist between means whose dark rectangles do not overlap. (A) Albinos, April 12 (from preserved specimens). (B) Albinos under laboratory conditions, May 5, 1952. (C, D, E) Albinos above and normals below, May 5, 1952. (C) Pool population. (D) Metal trough. (E) Concrete trough. Mean weight in grams is given to right of each sample.

TABLE 1. *Survival of albino and normal spadefoot toad larvae*

		Number	Survival	Chi-square of difference
Pool	Albino	100	12	17.0
	Normal	100	38	
Metal trough	Albino	100	91	.48
	Normal	100	88	
Concrete trough	Albino	46	42	.04
	Normal	23	22	
Gallon jar	Albino	12	12*	—

* Includes three accidental deaths caused by handling.

but were about equally distributed in the two sections at the conclusion of the experiment. Very little algal growth was apparent. The water was clear. During the morning a good part of this trough was shaded by nearby oaks. Predation here was as unlikely as in the metal trough.

The third control consisted of a group of twelve albinos, kept in a gallon jar of tap water in the laboratory with *Anacharis* as food. The water temperature varied only slightly.

Experimental Results

From the beginning of the experiment on April 12, the pool and controls were observed several times a week. On April 30 the population of the pool appeared unchanged. *The next day,* May 1, a marked reduction in the number of tadpoles was evident and the mud around the edge of the rapidly receding pool was now covered with tracks of the raccoon (*Procyon lotor*). Two days later, on May 3, the experiment was concluded and all remaining larvae and metamorphosed young in the pool and controls were caught, weighed, measured, and counted.

In the pool situation where 100 each of the albino and normal forms had been exposed to predations for 21 days (April 12 to May 3), 38 normals and 12 albinos survived (table 1). Of these, 19 normals and one albino had metamorphosed and were found buried in the sand around the fence; the remainder (19 normals and eleven albinos) were still in the larval stage. The Chi-square value was 17.0, showing a statistically significant difference in survival between the normal and albino spadefoots. No significant differences in survival were found in the controls. Although no raccoon was seen, it can logically be inferred that the sudden removal overnight of about 150 larvae was due to the activities of this carnivore.

Developmental Differences

My inability to duplicate the environmental conditions of the experimental pool resulted in differences in developmental rates which have bearing on the survival of albino forms in nature. The water level of the pool dropped rapidly

TABLE 2. *Survival through metamorphosis of 20 albino and 20 normal spadefoot toads*
(Note later development of the albinos)

Date	Albino	Normal
1952		
May 13	0	2
14	0	8
15	6	8
16	7	1
17	4 (1 dead)	1
18	2	0
19	1	0
Total	20	20

TABLE 3. *Water temperature in degrees Fahrenheit*

	May 4— 12:30 p.m. P.S.T.	May 12— 4:45 p.m. P.S.T.
Pool	89	dry
Metal trough	69	82
Concrete trough	67	76

during the course of the experiment and dried up shortly thereafter. The smaller body of water in the pool showed greater variation in water temperatures than did the controls (table 3). The water was not analyzed, but as the pool became smaller it is assumed that the mineral content and pH changed, factors shown by Burgess (1950) to influence the rate of developing spadefoot toad larvae. Because of these and other factors, all of the tadpoles in the pool metamorphosed earlier and at a small size than those in the contols (fig. 2). None of the individuals in the control situations noticeably approached metamorphosis, as indicated by the lack of front legs, yet they were significantly longer and weighed more than those from the pool. *In every case* and in all situations, the albinos were smaller and less developed than the companion normal individuals; hence selective factors other than the relation of predation to skin color would operate against the albinos.

SURVIVAL THROUGH METAMORPHOSIS

It has been noted that, because of the differences in environmental conditions, none of the control animals developed to the stage of metamorphosis. As this stage would be critical in survival of albinos versus normals, an attempt was made to test whether any difference in mortality occurred at the time of metamorphosis. From tadpoles remaining in the concrete trough (table 1) a sample of 20 of each form was taken. It was impossible to obtain animals in the same stage of development, as all the normal forms were noticeably more advanced than the albinos. These were placed in separate aquaria outside the laboratory. Metamorphosis was considered to have been completed successfully when the young toad left the water and buried itself in the sand. Results of this phase of the experiment are summarized in table 2. All but one albino survived, and they were vigorous for several days after metamorphosis. The albinos, however,

FIG. 3. Albino larva of the spadefoot toad (*Scaphiopus h. hammondii* Baird) used in this experiment. 2 × natural size. Photo by N. Cohen.

were slower to metamorphose. From these data there appears to be no significant difference in survival between the two forms during metamorphosis.

An attempt was made to bring samples of each form through the year for breeding experiments. By June 12, about 28 days following metamorphosis, only five of the more than 50 metamorphosed albinos remained alive. These, however, were shriveled and lethargic, and by June 21, all the albinos had died. Twenty normals were still alive and healthy under the same conditions.

Discussion

We can only assume that the albinism dealt with is of a genetic nature. The metamorphosed young did not live for breeding experiments. No differences in the pool population could be discerned which might possibly have indicated heterozygosity. Concerning the parentage of the larvae, it can only be said that the population in the original pool (298 albinos and 1,154 normals) was derived from more than one mating, probably three to five females were involved. Stebbins (1950) gives the figure of 300 to 500 eggs laid per female. Mortality among the larvae may have been large before the pool was discovered as this species is noted for its cannibalistic habits.

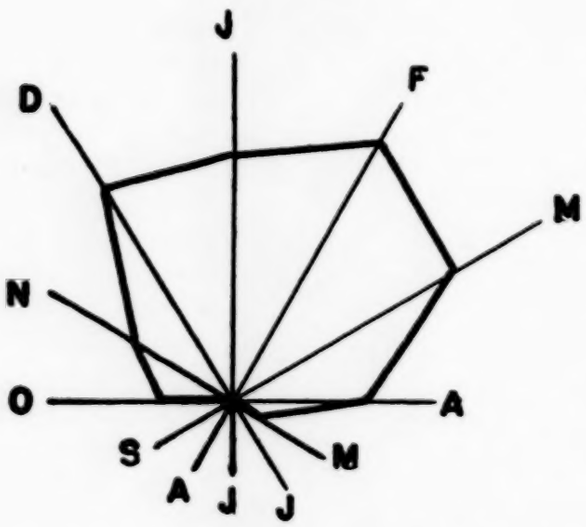

Fig. 4. Seventeen-year mean precipitation diagram, 1934–1951, San Joaquin Experimental Range, O'Neals, Madera County, California. 1 cm. = 1 inch

The form of albino dealt with here is considered a true albino (fig. 3), having pink eyes, although other white forms occur in this area.

A description of the area, its physical features and weather, is given by Hutchison and Kotok (1942). Of particular significance to this study is the seasonal pattern of precipitation. Its distribution (fig. 4) is such as to cause a rapid drying in late spring, resulting in the extremes observed in the semi-arid parts of the non-irrigated portion of the San Joaquin Valley. Spadefoot toads are adapted to these conditions so that yearly variations in rainfall in the spring are compensated for in the response of the developing larvae to the changing quantity of the water. Thus slow development of albino individuals is a factor which would further act to eliminate them under these conditions regardless of their color.

Summary

A population of 1,452 spadefoot toad larvae in a vernal pool contained 20% (298) of albino individuals. The evidence presented indicates that predation by the raccoon on a population of 200 larvae (100 normals, 100 albinos) left in a natural pool is probably responsible for a statistically significant reduction of the albino form. The Chi-square value for this difference is 17.0. No difference in survival through early metamorphosis was found between normals and albinos in the controls, but development in the albino form was slower in every situation studied. The albino form did not survive for breeding experiments.

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CHROMOSOMAL TRANSLOCATIONS IN INTERSPECIFIC HYBRIDS OF THE GENUS GOSSYPIUM

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In the past cytologists have made considerable contributions toward an understanding of the systematics of the genus Gossypium. Especially, Skovsted (1933–1937), Webber (1934–1939) and Beasley (1940–1942) have demonstrated the sharp cytological divisions existing between the different sections of the genus, namely: Anomala in Africa and Arabia, Stocksiana in Arabia, Sturtiana in Australia, Wild American, and the cultivated cottons of the Old World, the Herbacea.[2] The amphidiploid origin of the last section, the Hirsuta, from which are derived the most important commercial cottons of today, was first demonstrated by cytological means (Skovsted, 1934 and Beasley, 1940).

The cytological relationships within these groups, while certainly not omitted from consideration by the above mentioned authorities, have not yet been completely clarified. Also, the search for those species in the various sections which might be closest to the connecting links between the neighboring sections poses a special challenge. Of course, the tools of the cytologist can be applied only to those hybrids in which there is conjugation between chromosomes; and the looser the pairing, the blunter become these tools. The present paper represents a study of metaphase associations in the pollen mother cells of some hybrids from the sections Herbacea, Anomala and Hirsuta which show a high degree of chromosome conjugation.

Plant Materials

Stocks of Asiatic cottons are listed together with their origins in tables 1 and 2. The identification numbers are those assigned by the source indicated. If strains have been mentioned in earlier publications references are given.

G. arboreum L. The strains from Yu and Chang and those from Silow were obtained by Dr. S. G. Stephens who brought them to North Carolina and was kind enough to make them available to the author. The line S 5000 was extracted by Stephens from the F_2 N14 (Silow) × C.K. 5–27 (Yu and Chang). Two of the Indian strains were collected by Mr. W. Koelz in India for the U. S. Department of Agriculture and made available through that agency. Okinawa stems from a single plant of unknown origin found growing on the Pacific Island of that name by an American soldier of World War II who collected seeds and placed them at the disposal of the U. S. Department of Agriculture. The strain from Burma was received from Mr. R. L. Cuany of the Empire Cotton Growing Corporation in the Anglo-Egyptian Sudan as race *burmanicum*.

G. herbaceum L. Mr. Cuany also sent us most of the strains of this species, which are listed in table 2 and arranged in accord with the taxonomic classifica-

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[2] These are commonly designated as Asiatic cottons. This term will be retained here though its use is misleading when applied to two species which are also widely distributed in Africa and one of which appears to be of African origin.

TABLE 1. *Modal chromosome behavior in hybrids between* G. arboreum *lines*

G. arboreum ♂		♀ (Okinawa, unless stated otherwise)
Origin and type	Identification	
China:		
Sunred spotless	C.K. 2–27 (Yu and Chang, 1948)	pollen fertile*
Red Margin Spotless	N.K. 1–26 (Yu and Chang, 1948)	13_{II}
Red Margin Spotted	C.K. 7–27 (Yu and Chang, 1948)	13_{II}
Chinese Pale	N44 (Silow, 1941)	pollen fertile
India and Pakistan:		
Koelz coll.	C.B. 2549 (U.S.D.A.)	pollen fertile
Koelz coll.	C.B. 2559 (U.S.D.A.)	13_{II}
Sanguineum	C.B. 787 (U.S.D.A.)	pollen fertile
Nanking	A_{2-15} (U.S.D.A.)	13_{II}
White flower, Ghost spot	N14 (Silow, 1941)	pollen fertile (Okinawa used as ♂)
var. *cernuum*, Garo Hill	A_{2-2} (U.S.D.A.)	13_{II}
Burma:	ANCB2 (Cuany)	tested only with *G. herbaceum*, see table 2
Synthetic Genotype:		
White flower, Basic spotless	S 5000 (Stephens)	pollen fertile

* Hybrids designated as pollen fertile were studied from pollen preparations only, the remainder also from metaphase smears.

tion of Hutchinson (1950). The accession numbers of the stocks listed as received from Cuany are those of the world collection of the Empire Cotton Growing Corporation. Two strains of *G. herbaceum* came from the collection made by Dr. J. R. Harlan for the U.S.D.A. in Turkey.

Mr. Cuany not only contributed a large collection of seeds but the author is also very much indebted to him for having made most of the crosses between *G. herbaceum* lines; this was necessary as *G. herbaceum* fails quite frequently to retain its bolls under North Carolina conditions.

C.B. 743 used in table 8 is a U.S.D.A. Station stock listed as *var. typicum*.

G. anomalum Wawra et Peyr.: C.B. 1051, U.S.D.A. stock.

TABLE 2. *Modal chromosome behavior of crosses with* G. herbaceum *lines*

G. herbaceum ♂			♀	
Origin and race		Identification	*Arboreum*	*Herbaceum*
1. South Africa:	*Africanum*	HA20 (Cuany)	$11_{II}+1_{IV}$ (1)*	13_{II} (*Wightianum*)*
2. North Africa:	*Acerifolium*	HFA11 (Cuany)	$11_{II}+1_{IV}$ (3)	
3. India:	*Wightianum*	HF1–2A	$11_{II}+1_{IV}$ (2)	13_{II} (Gillett's Iraqui)
4. Levant:	*Persicum*	HTP8 (Cuany)		13_{II} (*Africanum, acerifolium*)
5. Levant:	(Harlan coll.)	P.E.I. 173703 (U.S.D.A.)	$11_{II}+1_{IV}$ (1, 2, 4)	
6. Levant:	(Harlan coll.)	C.B. 2534 (U.S.D.A.)	$11_{II}+1_{IV}$ (1)	
7. Levant:	(Gillett's Iraqui)	HTP8B (Cuany)	$11_{II}+1_{IV}$ (2)	
8. Levant:	(Tashkent cream pollen)	HTP15 (Cuany)	$11_{II}+1_{IV}$ (1, 2)	13_{II} (*Kuljianum*)
9. China:	*Kuljianum*	HTK1 (Cuany)	$11_{II}+1_{IV}$ (1)	

* Types used as female parents are given in parentheses. For *G. arboreum* these were 1, Okinawa; 2, *Sanguineum;* 3, Burma; 4, White flower, ghost spot.

G. hirsutum L.: The Stoneville line is derived from a haploid doubled with colchicine in the nineteen-thirties by Beasley and maintained by grafting. Lacandon is a perennial *G. hirsutum* (race *latifolium* Hutchinson) collected by Dr. Gustav Stromsvik, Carnegie Institution, for Dr. S. G. Stephens among the Lacandon Indians in the Upper Usumacinta region of Central America.

Most Stoneville-Asiatic hybrids were obtained through the kindness of Dr. James R. Meyer who had produced them by means of embryo culture and sent scions to Raleigh, N. C., where they were grafted on commercial cottons.

Laboratory Techniques

The purpose of the laboratory analyses was to find out whether a given strain or hybrid possessed reciprocal translocations. Two techniques were employed for that purpose:

1) Young anthers were fixed in Bradley's mixture (1948) of one part glacial acetic acid to three parts of absolute ethyl alcohol to four parts of chloroform. The next day they were either transferred to

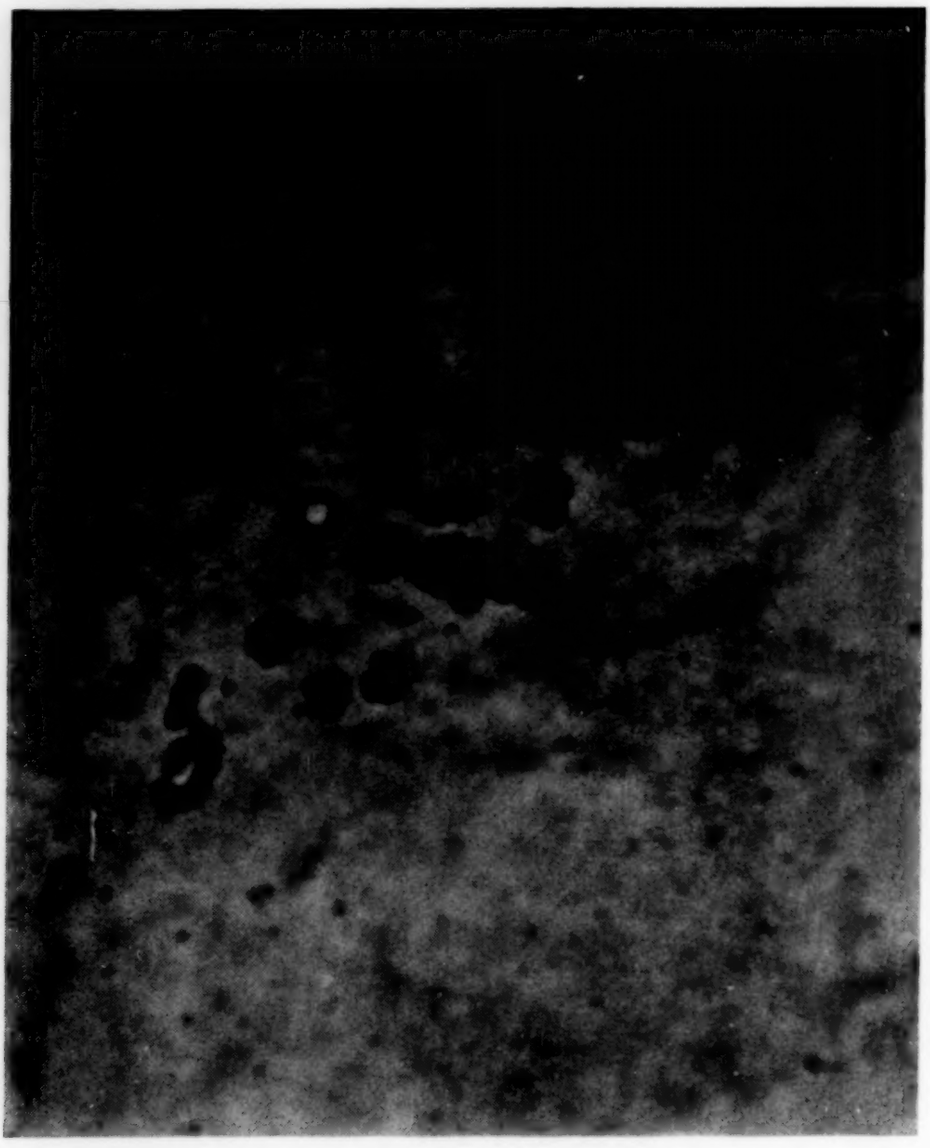

Fig. 1. Eleven bivalents and one ring of four chromosomes in a pollen mother cell of *G. arboreum* race *sanguineum* × *G. herbaceum* race *Wightianum*. Late diakinesis. Microphotograph ca. 2150 ×.

80% alcohol for storage or squashed in 65% acetic acid saturated with carmine, covered and examined.

2) Flowers were gathered in the morning before anthesis, brought to the laboratory and some time after anthesis several anthers were placed in a drop of lactophenol stained with acid fuchsin. The pollen grains were rinsed in that mixture, the empty anthers removed and the preparation covered. All grains on a slide were classified as either empty of contents or as filled. All the interspecific hybrids were analyzed by methods 1 and 2, as were also a number of hybrids made between strains of the same Asiatic species. A number of additional intraspecific hybrids were only tested for semisterility by method 2.

Vigorously growing plants having less than 10% empty pollen grains were classified as pollen fertile and those with more than 30% empty grains as semisterile.

From each of the Asiatic crosses three plants were grown and subjected to pollen analysis (in a few cases only two plants survived), while the chromosome associations were mostly studied on a single representative.

Results

G. arboreum × *G. herbaceum*

All the available lines of *G. arboreum* were found to possess the same arrangements of their chromosomes, i.e., their hybrids formed 13 pairs at meiosis (table 1). The same was true for the lines of *G. herbaceum*, while all hybrids between the two species showed one figure of four chromosomes in the majority of their pollen mother cells indicating heterozygosity for one reciprocal translocation (tables 2, 3; fig. 1). Morphological differences between the various lines were large enough in all cases to show that the supposed interracial and interspecific hybrids were in fact true F_1 plants.

The translocation figure was arranged either as a ring or as a chain of four chromosomes; chains of three chromosomes and one single also occurred. In these rings and chains the centromeres of either adjacent or alternate chromosomes pointed to the same pole. Besides the translocation figure no other cytological aberrations were evident in the meiotic metaphase of the interspecific hybrids. There was little indication that conjugation was less in these hybrids as they were not found to be inferior to pure *G. arboreum* in the number of chiasmata formed per pollen mother cell (table 3). For a comparison mean chiasma frequencies per cell were calculated by considering only those points where two chromosomes were clearly joined. Nonterminalized chiasmata were rare; they were arbitrarily counted as singles as it was not possible to distinguish between one or more chiasmata in the paired arms in which they occurred. As similar criteria were applied to interspecific and interracial hybrids any bias introduced by this method should not be serious. It should be mentioned that for practical reasons the chiasma counts had to be gathered from all the lines used in compilation of tables 1 and 2 throughout two summer seasons. During this protracted period the environmental conditions were far from uniform.

TABLE 3. *Percentage frequencies of metaphase configurations in Asiatic species and species hybrids*

	Percentage frequency in	
	G. arboreum × *herbaceum*	*G. arboreum* (Interracial hybrids)
$11_{II}+1_{IV}$	81.75	—
$11_{II}+1_{III}+1_{I}$	3.97	—
$10_{II}+1_{IV}+2_{I}$	2.38	—
13_{II}	7.94	82.81
$12_{II}+2_{I}$	2.38	12.50
$11_{II}+4_{I}$	1.58	3.13
$10_{II}+6_{I}$	—	1.56
Cells counted	126	64
Mean chiasma frequency per cell	22.88 ± 0.26	22.26 ± 0.33

TABLE 4. *Fertility of* G. arboreum *and* G. arboreum × herbaceum

Parent strains		Total seeds counted (o.p.)	% aborted seeds ("Motes")	Total pollen counted	% empty pollen grains
G. arboreum	*G. herbaceum*				
Okinawa (selfed)	—	191	10.5	1420	3.1
Okinawa	×C.B. 2534	206	16.5	2062	44.9
Okinawa	×*Kuljianum*	165	13.3	2187	45.0
Sanguineum (selfed)	—	224	3.6	1391	0.9
Sanguineum	×Gillett's Iraqui	209	3.8	1941	43.8
Sanguineum	×Tashkent cream pollen	205	4.4	4048	43.3

It may appear surprising that the translocation feature has been so widely overlooked by previous students of Asiatic cotton. This could be explained by the fact that most workers had been mainly concerned with female fertility, which, however, is as good in the hybrids as in pure *G. arboreum* as shown in table 4. To make certain that the seeds had been accurately scored 130 "good" seeds from each of the six entries in table 4 were germinated on filter paper. Their germinability proved about the same (4.6% non-germinants from the two *G. arboreum* strains and 4.0% from the four hybrids).

Only a microscopic analysis of pollen grains followed up by a study of meiosis could have revealed the existence of the abnormality. In table 4 are listed the percentages of empty pollen grains of the *G. arboreum* lines Okinawa and *sanguineum* and of some of their hybrids with *G. herbaceum* lines. While *G. arboreum* showed only a small degree of abortion the hybrids between the two species exhibited semi-sterility, as is characteristic of translocation heterozygotes. It should be noted that these abortion frequencies were only rough approximations as it was evident that in this material the values were influenced by environmental conditions; the relationship is not yet clear. It should be added that it is probable, though not certain, that the pollen abortion feature is caused by the translocation as both occur only in the interspecific hybrids. Factors other than the translocation might feasibly affect fertility, while translocation

TABLE 5. *Metaphase conjugation in* G. anomalum × G. herbaceum

	Number of pollen mother cells		
	G. herbaceum parent used		
	Wightianum	*Kuljianum*	Total
13_{II}	3	15	18
$12_{II}+2_{I}$	4	13	17
$11_{II}+4_{I}$	1	8	9
$10_{II}+6_{I}$	—	6	6
$10_{II}+1_{III}+3_{I}$	—	1	1
$9_{II}+8_{I}$	—	1	1
	—	—	—
Totals	8	44	52

Mean number of chiasmata per cell: 16.62 ± 0.41

TABLE 6. *Metaphase conjugation in* G. arboreum × G. anomalum

	Number of pollen mother cells		
	White flower, ghost spot × *G. anomalum*	*Sanguineum* × *G. anomalum*	Total
13_{II}	2	1	3
$12_{II}+2_{I}$	1	—	1
$11_{II}+1_{IV}$	4	4	8
$11_{II}+1_{III}+1_{I}$	1	2	3
$11_{II}+4_{I}$	1	1	2
$10_{II}+1_{IV}+2_{I}$	5	3	8
$10_{II}+1_{III}+3_{I}$	2	—	2
$9_{II}+1_{IV}+4_{I}$	1	1	2
$9_{II}+1_{III}+5_{I}$	—	1	1
$9_{II}+8_{I}$	1	—	1
$8_{II}+1_{III}+7_{I}$	1	—	1
$5_{II}+1_{III}+13_{I}$	1	—	1
$4_{II}+1_{IV}+14_{I}$	1	—	1
	—	—	—
Totals	21	13	34

Mean number of chiasmata per cell: 17.12 ± 0.68

TABLE 7. *Metaphase conjugation in* G. hirsutum × G. herbaceum

	Number of pollen mother cells		
	Stoneville ×C.B. 743	Stoneville ×Gillett's Iraqui	Total
$9_{II}+2_{IV}+13_{I}$	16*(3)	22*(9)	38
$8_{II}+2_{IV}+1_{III}+12_{I}$	4*(1)	2	6
$7_{II}+2_{IV}+2_{III}+11_{I}$	1*(1)	—	1
$9_{II}+1_{IV}+1_{V}+12_{I}$	1*(1)	—	1
Totals	22	24	46

*(3) Figures behind asterisks indicate the number of cells in which less than the specified number of univalents could be seen. This could be due to loss of peripheral areas of the cells or to covering of univalents by other chromosomes.

heterozygotes without sterility are known in other diploid species (Thompson and Hutcheson, 1942).

Hybrids of Asiatic cottons with G. anomalum

Obviously, the differences in chromosome arrangement between the two Asiatic cottons must be reflected in the hybrids of these two species with any third one, provided that the number of chiasmata is sufficiently large to permit the formation of multivalents. Tables 5 and 6 show the extent of metaphase conjugation in the hybrids between the Asiatic cottons and *G. anomalum*. (Hybrids between *G. herbaceum* and *G. anomalum* had to be made with the latter as the female parent, as indicated in the heading of table 5 because *G. herbaceum* holds its capsules only rarely under Raleigh, N. C. conditions, while some *G. arboreum* lines make good seed parents).

Practically no multivalents were formed in the *G. herbaceum* hybrids (table 5) but only pairs or pairs and univalents, which indicated that the hybrids were not heterozygous for a translocation. On the other hand, in the hybrids between *G. arboreum* and *G. anomalum* (table 6) $11_{II}+1_{IV}$ and $10_{II}+1_{IV}+2_{I}$ were the modal types and one quadri- or trivalent occurred in most cells; these hybrids, therefore, were heterozygous for a reciprocal translocation. The absence of rings in the *G. herbaceum* hybrids and their presence in those with *G. arboreum* could not have been due to a lower degree of conjugation in the latter, as a comparison of chiasma frequencies at the bottom of tables 5 and 6 shows.

Hybrids between tetraploid G. hirsutum *and the Asiatic cottons*

In New World-Asiatic cotton hybrids the Asiatic chromosomes associate with 13 of the New World chromosomes of presumably Asiatic origin. The remaining 13 chromosomes from the tetraploid are homologues of the Wild American species chromosomes and form univalents in the hybrids, or, very occasionally, conjugate with those of Asiatic origin. This had been well established by the several authors listed below under Discussion.

TABLE 8. *Metaphase conjugation in* G. hirsutum × G. arboreum

	Number of pollen mother cells			
	Stoneville × C.K. 7-27	Stoneville × Garo Hill	Lacandon × Okinawa	Total
$8_{II}+1_{IV}+1_{VI}+13_{I}$	13*(2)	12*(1)	16*(3)	41
$8_{II}+1_{III}+1_{VI}+14_{I}$	1*(1)	—	1	2
$7_{II}+1_{IV}+1_{VI}+1_{III}+12_{I}$	1	—	—	1
$9_{II}+2_{IV}+13_{I}$	—	1	—	1
$9_{II}+1_{IV}+1_{V}+12_{I}$	1	—	—	1
$9_{II}+1_{V}+16_{I}$	—	—	1	1
$10_{II}+1_{VI}+13_{I}$	1	—	1	2
Totals	17	13	19	49

*(2) see footnote table 7.

It still remained to be seen what, precisely, the differences were in crosses between the New World cottons and each one of the Asiatic species.

The most frequent type of metaphase plates found in these hybrids were $9_{II} + 2_{IV} + 13_{I}$ for *G. hirsutum* × *herbaceum* (table 7) and $8_{II} + 1_{IV} + 1_{VI} + 13_{I}$ for *G. hirsutum* × *arboreum* (table 8). Clearly, the two types of hybrids differed by one translocation, the *G. herbaceum* hybrids having two configurations of four chromosomes and the hybrids with *G. arboreum* having one ring or chain of four and one of six chromosomes. The picture is complicated by some cells which differed from the modal types. Such cells can be found in both tables 7 and 8, but they are relatively rare and may be interpreted as being due to either conjugation of Wild American chromosomes (e.g., table 8, row 3) or to failure of conjugation between Asiatic chromosomes (table 8, row 4).

Discussion

Comparison of results with previous authors

This work was originally stimulated by an apparent contradiction between the findings of Skovsted (1933) and Webber (1935, 1939) on one hand and Beasley (1942) on the other. Beasley reported the occurrence of one association of four chromosomes in a hybrid between *G. arboreum* and *G. herbaceum* whereas the former two authors found no such evidence for the existence of a reciprocal exchange of segments in their hybrids between the two species. The writer thereupon studied meiosis in two different *G. arboreum* × *herbaceum* hybrids kindly placed at his disposal by Dr. C. L. Rhyne, Jr. and discovered that both possessed a ring of four chromosomes in addition to 11 bivalents in a large proportion of their metaphasic pollen mother cells. It was decided to make a survey of the available lines of the two species and to explore whether a given arrangement was the same for all lines in each of them. This was found to be true and all the available lines of *G. herbaceum* formed 13 pairs in their pollen mother cells, as did all lines of *G. arboreum* while they differed from each other by one translocation (tables 1–2). The present survey was limited to metaphase preparations and therefore inversions and other chromosomal differences which cannot be seen in the condensed metaphasic units were not observed though they probably exist. (Beasley (1942) mentions the occurrence of a bridge with fragment in the hybrid between the two species, and Sikka, Khan and Afzal (1945) observed differences in the number of satellites in Asiatic cottons.) From the data in table 3 it would appear, that apart from the translocation and on the whole, the chromosomes of the two species are homologous as the extent of pairing is similar in the hybrid and in *G. arboreum*.

The finding that the number of chiasmata is the same in the hybrid as in one of the parents is in contradiction to Skovsted's (1933) and Webber's (1935) remarks that the chiasma frequency is lowered in the hybrid. As the ascertaining of chiasma frequencies is notoriously difficult in material with chromosomes as small as those of the cottons and is presumably also subject to environmental modification the results might not always apply. However, the finding that there is no difference in chiasma frequency falls in line with the author's observation that amphidiploids 4n *G. arboreum-herbaceum* have so far exhibited no preferential affinity between the chromosomes of the parent species, but random conjugation. This is borne out by the autotetraploid ratios produced in the progeny of these amphidiploids (Gerstel, in preparation). Such an argument, of course, is valid only for those chromosomes which were genetically marked with segregating alleles in the amphidiploid material, viz., Y_a, R_2 *and* Rl_a, a new complementary, *red lethal.*

On the other hand, Silow (1944) measured a small but significant reduction in cross-over frequency for the Lc_1-L linkage group in the interspecific hybrid which would indicate some difference in that chromosome sector.

Hybrids between the Asiatic cottons and the truly wild and presumably more primitive *G. anomalum* have also been studied before by a number of authors. In these hybrids conjugation is less complete than in the *G. arboreum* × *herbaceum* crosses and univalents are fairly frequent. Therefore, a lower proportion of cells will exhibit the evidence for translocations in the *G. anomalum* hybrids than in the hybrids between the Old World cottons. In *G. arboreum* × *anomalum* hybrids of Webber (1939) and Afzal, Sikka and Ahsan-ul-Rahman (1945) multivalents were not seen at all. Iyengar (1942) observed occasional tri- and quadrivalents in similar material. Skovsted (1937) found one-third pollen mother cells with either a trivalent or a quadrivalent in *G. arboreum* × *anomalum* crosses and no multivalents in a *G. herbaceum* × *anomalum* hybrid. He illustrated this difference in figures 3 and 4. Unfortunately, the data from the two kinds of crosses were combined in Skovsted's summary table under "Asiatic cottons × *G. anomalum*" and the distinction was lost.

Of the long list of investigators who have studied New World-Asiatic cotton hybrids in the past the earlier papers by Baranov (1930), Zhurbin (1930), Nakatomi (1931) and Feng (1935) reported generally $13_{II} + 13_I$ at meiosis. However, some configurations in Nakatomi's illustrations might represent multivalents; he and the others may have interpreted quadrivalents as overlapping pairs. Longley (1933) and Webber (1935) studied the same natural hybrid between an undetermined Asiatic cotton species and a New World cotton, presumably *G. hirsutum* according to Webber. While Longley did not mention occurrence of any multivalents, Webber found one or more quadrivalents in 12 out of 62 pollen mother cells; four quadrivalents were discerned in two of them. Iyengar (1944) observed one to two quadrivalents or trivalents in most plates of a *G. hirsutum* × *herbaceum* hybrid. His data are readily reconciled with the two translocations found here for *G. hirsutum* × *herbaceum*, though in his case the amount of conjugation was either less, or his tendency to resolve "overlaps" greater. Skovsted's (1934) results are also similar to those reported here for in a hybrid between *G. barbadense* and *G. arboreum* he counted 5 cells with $8_{II} + 1_{IV} + 1_{VI} + 13_I$ (fig. 7, loc. cit.). Thirty cells show a lower amount of conjugation than this; five plates showed excess pairing. Beasley (1942) studied three New World-*herbaceum* combinations and in all of them $9_{II} + 2_{IV} + 13_I$ was the modal number, as it was in the present report for similar hybrids.

Phylogenetic considerations

Judgment in matters of phylogeny should never be based on a single character and must wait for corroboration from additional sources. It is well known that in some species chromosomal arrangements are very labile and various translocations may occur even in a single population (e.g., Walters, 1942; Lewis, 1950). If such were the case in Gossypium no phylogenetic value at all could be attributed to the translocations. However, it appears that translocations are of very rare occurrence in this genus. The widely spread Asiatic cottons seems to have configurations typical for each one, with no translocations known within either species. The Wild American species represent a number of endemics scattered over a wide area and highly differentiated morphologically; those that can be crossed form generally 13 pairs and no quadrivalents in their hybrids (Webber, 1935; Skovsted, 1937; and author's unpublished data) and in hybrids with New World cotton (reviewed

by Brown and Menzel, 1952). Thus no translocation has been formed in the evolution of the D[3] chromosome set of either wild or cultivated American species. As far as can be judged by present records the three amphidiploid species *G. hirsutum, G. barbadense* and *G. tomentosum* form 26 pairs in their hybrids (Webber, 1935, 1939; Skovsted, 1937). More critical information, especially on *G. tomentosum* hybrids, would be desirable.

Thus, the *G. arboreum* × *herbaceum* translocation stands alone, with the exception of the described "swarm" of translocations in the A set of the New World cottons, which might well have arisen together with, or shortly after, the doubling of the chromosomes of the ancestral allopolyploid.

Merely for the purpose of furthering discussion a phylogenetic tree may be constructed for the B, A and AD genoms. There is wide agreement that *G. anomalum* is the primitive species in this group (reviewed by Hutchinson, Silow and Stephens, 1947; and Wouters, 1948). While there is no definite proof that *G. herbaceum* var. *africanum* is a truly wild form and not an escape from cultivation it could easily be the most primitive type of the species as plant morphology (Chevalier, 1936) and chromosome morphology (Sikka, Khan and Afzal, 1944) seem to indicate. As this form of the species is of purely African distribution it appears probable that the origin of *G. herbaceum* was in Africa. Unfortunately, the chromosomes of no truly primitive form of *G. arboreum* have been investigated as yet, but the similar arrangement of the chromosomes of all the types which were studied from a wide geographical range would indicate that this arrangement is typical for the species and that the translocation difference between *G. herbaceum* and *G. arboreum* might be regarded as a taxonomic criterion.

The cytological evidence suggests that *G. herbaceum* is closer to the tetraploid New World cottons than *G. arboreum*. Interesting here are the anatomical studies by Abraham and Ayyar (1938). They observed a lower point of separation of the vascular strands in the flowers of the New World cottons and *G. herbaceum* than in those of *G. arboreum*, and opined that "the similarity in the stages of forming the anatomical patterns in the *herbaceums* and the *hirsutums* suggest that the former has a greater probability of being the Asiatic parent than the *arboreums*." Further study of anatomical features of parent species and of their combination in artificial amphidiploids should be rewarding.

The possibility that a species apparently centered in Africa may be closer to the New World cottons than the now largely Asiatic *G. arboreum* should be taken in account in the further discussion of the origin of the New World amphidiploids. It might be considered to weaken the hypothesis of a recent Pacific transfer of an Asiatic component of the amphidiploid hybrids, which was tentatively pronounced by Hutchinson, Silow and Stephens (1947). The possibility of an Atlantic transfer should perhaps not be entirely neglected, though the distributional pattern of New World cottons speaks against it (Hutchinson, Silow, and Stephens, 1947). There is, of course, the alternative that the amphidiploid cottons may be of early Tertiary age as postulated by Stebbins (1947) and that the modern world distribution of the genus bears little relation to their phylogeny.

Summary

1. Hybrids between the cultivated cottons of the Old World, *G. arboreum* and *G. herbaceum*, form eleven bivalents and one figure of four chromosomes at meiotic metaphase.

[3] Following the letter designations introduced by Beasley (1940): A for Asiatic, B for *G. anomalum*, D for wild American and AD for cultivated New World genomes.

2. Hybrids between *G. arboreum* and *G. anomalum*, a wild African species, also exhibit one figure of four in many cells, while *G. anomalum* × *G. herbaceum* hybrids form no multivalents.

3. The amphidiploid New World cotton, *G. hirsutum*, gives two figures of four in crosses with *G. herbaceum* and a figure of four and a figure of six in hybrids with *G. arboreum*.

4. Previous authors, notably Beasley and Skovsted, have observed the translocation figures in some of the hybrids but have failed to recognize their systematic occurrence.

5. Phylogenetic conclusions based on a single criterion may be very misleading, but the writer could not resist the temptation to speculate on some evolutionary relationships.

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PRESENT DISTRIBUTION AND HABITATS OF THE CONIFERS AND TAXADS

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INTRODUCTION

The distribution of conifers and taxads has long been known to have a striking association with the Pacific basin (Engler, 1926; Buchholz, 1948). The majority of the relic, endemic or disjunct genera concentrate on lands bordering both the east and west of the Pacific, in both the Northern and Southern Hemispheres. Among the polytypic and wide-spread genera, in practically all cases, the number of species is larger in regions bordering the Pacific than elsewhere. In order to seek an explanation of this striking phenomenon in plant geography, it seems desirable to study the correlation of the ranges of these genera with their habitats.

CLASSIFICATION AND GENERAL DISTRIBUTION

The existing genera of conifers and taxads are either primarily of the Northern or the Southern Hemispheres. Occasional outlying species may transcend the tropics, but the ranges of all genera quite distinctly belong to either the Northern or the Southern Hemispheres as can be seen from the following table which gives, in taxonomic sequence, a brief summary of the range of each genus. The scheme of classification follows mainly that given by Janchen (1950); with some modifications in the Cupressaceae by the present author (Li, 1953). In giving the figures for the number of species for each genus, notable recent publications of new genera and species, such as Buchholz (1949) and Li (1952, 1953), are taken into consideration.

Order Taxales
- Cephalotaxaceae
  - *Cephalotaxus* (6 spp.—Eastern Asia).
- Taxaceae
  - *Amentotaxus* (4 spp.—Eastern Asia).
  - *Torreya* (6 spp.—Eastern Asia and western and southeastern North America).
  - *Austrotaxus*[1] (1 sp.—New Caledonia).
  - *Nothotaxus* (1 sp.—Eastern China).
  - *Taxus* (7 spp.—Widely distributed in Europe, Asia, and North America).
- Podocarpaceae
  - *Phyllocladus* (6 spp.—New Zealand, Tasmania, New Guinea to Malaysia).
  - *Pherosphaera* (2 spp.—Tasmania and southeastern Australia).
  - *Microcachrys* (1 sp.—Tasmania).
  - *Saxegothaea* (1 sp.—Southern Chile).
  - *Acmopyle* (3 spp.—New Caledonia and Fiji).
  - *Dacrydium* (Ca. 20 spp.—Chile, New Zealand, Tasmania to Malaysia).
  - *Podocarpus* (Ca. 70 spp.—Widely distributed in the Southern Hemisphere and the tropics, extending north to Cuba and Mexico in America and to southern Japan and central China in Asia).

Order Pinales
- Cupressaceae
  - Subfamily Callitroideae
    - *Actinostrobus* (2 spp.—Western Australia).
    - *Callitris* (Ca. 20 spp.—Australia, Tasmania and New Caledonia).
    - *Fitzroya* (1 sp.—Southern Chile).
    - *Neocallitropsis* (1 sp.—New Caledonia).
    - *Octoclinis* (1 sp.—Southeastern Australia).
    - *Widdringtonia* (5 spp.—South Africa and southeastern Tropical Africa
    - *Diselma* (1 sp.—Tasmania).
    - *Papuacedrus* (3 spp.—New Guinea, Moluccas).
    - *Pilgerodendron* (1 sp.—Southern Chile).
    - *Libocedrus* (6 spp.—Southern Chile, New Zealand, New Caledonia).
    - *Tetraclinis* (1 sp.—Morocco, Algeria, Tunisia).

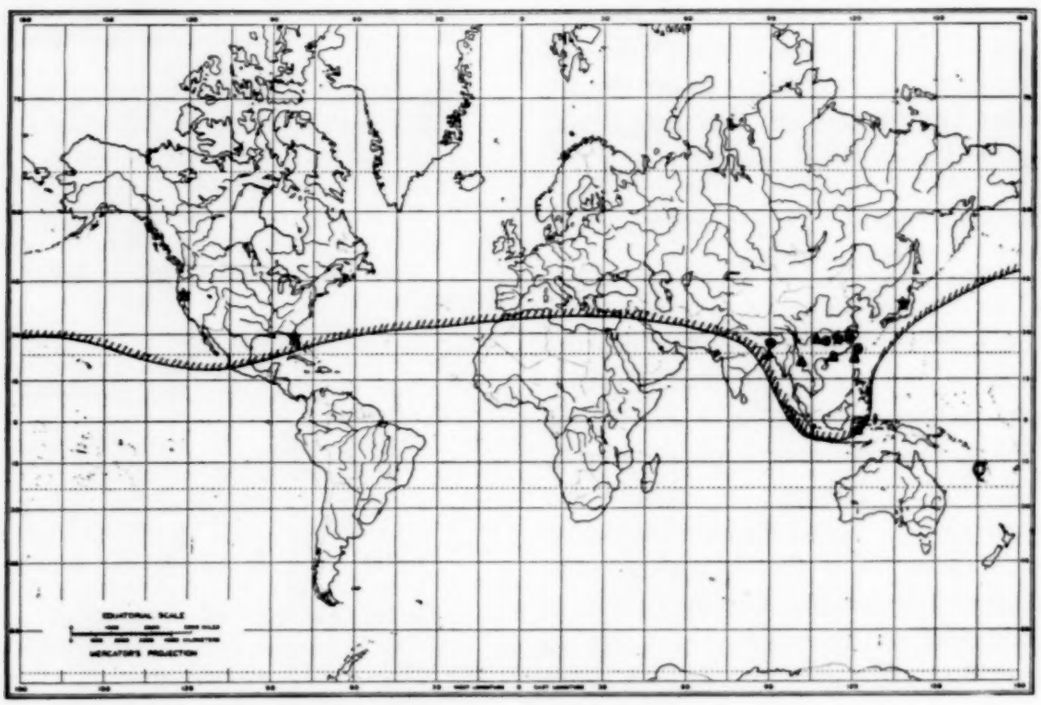
[1] *Austrotaxus* is of doubtful taxonomic status. Perhaps it should be referred to the Podocarpaceae as discussed further in the text.

Subfamily Cupressoideae
Cupressus (Ca. 12 spp.—North Africa, Asia to the eastern Mediterranean).
Chamaecyparis (Ca. 6 spp.—North America, Japan, Formosa).
Fokienia (1 sp.—Southeastern China to Tonkin).
Thujopsis (1 sp.—Japan).
Thuja (5 spp.—Eastern Asia and North America).
Biota (1 sp.—Northeastern Asia).
Heyderia (3 spp.—Pacific North America, Formosa, Hainan, southwestern China to northern Burma).
Arceuthos (1 sp.—Europe to western Asia).
Juniperus (Ca. 60 spp.—Widely distributed in the Northern Hemisphere).

Taxodiaceae
Sciadopitys (1 sp.—Japan).
Cunninghamia (2 spp.—China, Formosa).
Taiwania (2 spp.—China, Formosa).
Athrotaxis (3 spp.—Tasmania).
Cryptomeria (1 or 2 spp.—China, Japan).
Glyptostrobus (1 sp.—Southeastern China).
Taxodium (3 spp.—Mexico, southeastern U. S. A.).
Metasequoia (1 sp.—Central China).
Sequoiadendron (1 sp.—California).
Sequoia (1 sp.—California).

Araucariaceae
Araucaria (Ca. 20 spp.—Temperate South America, eastern Australia, New Caledonia, New Guinea).
Agathis (Ca. 20 spp.—Malaysia to New Hebrides, New Caledonia, Fiji and New Zealand).

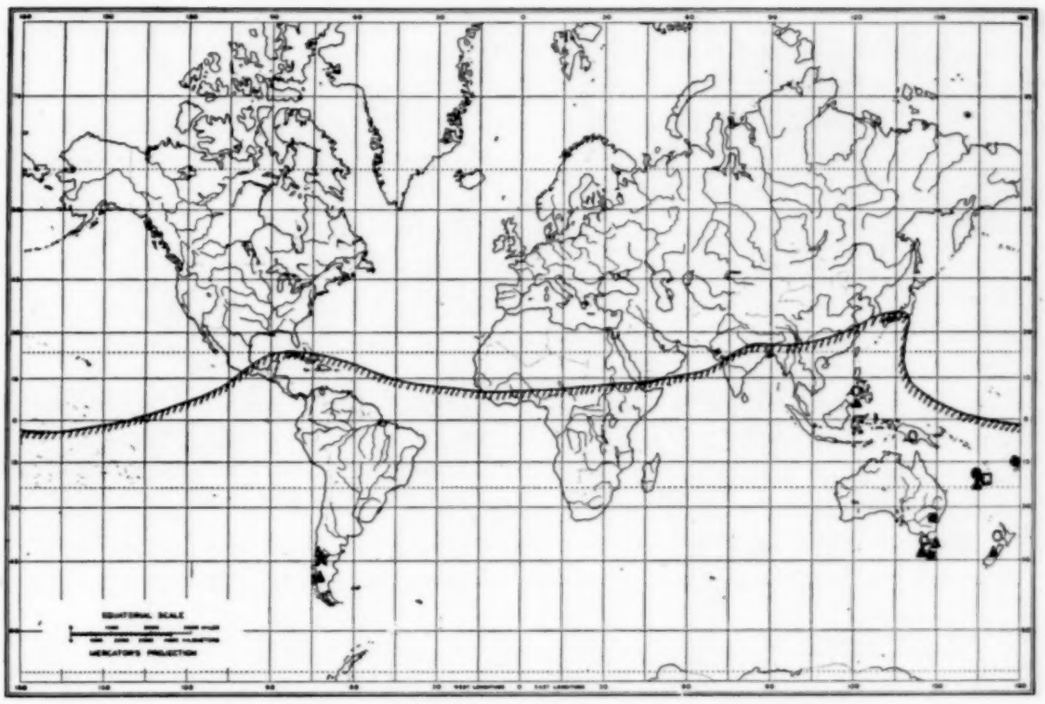
Abietaceae
Keteleeria (4 spp.—Southern China, Formosa).
Abies (Ca. 40 spp.—Widely distributed in temperate mountains and colder parts of the Northern Hemisphere).
Pseudotsuga (6 spp.—Eastern Asia and western North America).
Tsuga (10 spp.—Eastern Asia and North America).
Picea (Ca. 10 spp.—Widely distributed in colder parts of the Northern Hemisphere).



MAP 1. Generalized distribution of Cephalotaxaceae and Taxaceae.

LEGEND

▲ AHENTOTAXUS
○ AUSTROTAXUS (SEE MAP 2)
● CEPHALOTAXUS
■ NOTHOTAXUS
★ TORREYA
/// APPROXIMATE SOUTHERN LIMIT OF TAXUS



MAP 2. Generalized distribution of Podocarpaceae.

Cedrus (4 spp.—Northwestern Africa, Asia Minor, and northwestern Himalayas).
Larix (11 spp.—Mountains and northern parts of the Northern Hemisphere).
Pseudolarix (1 sp.—Eastern China).
Pinus (Ca. 80 spp.—Widely distributed in the Northern Hemisphere extending to the equator in southern Asia).

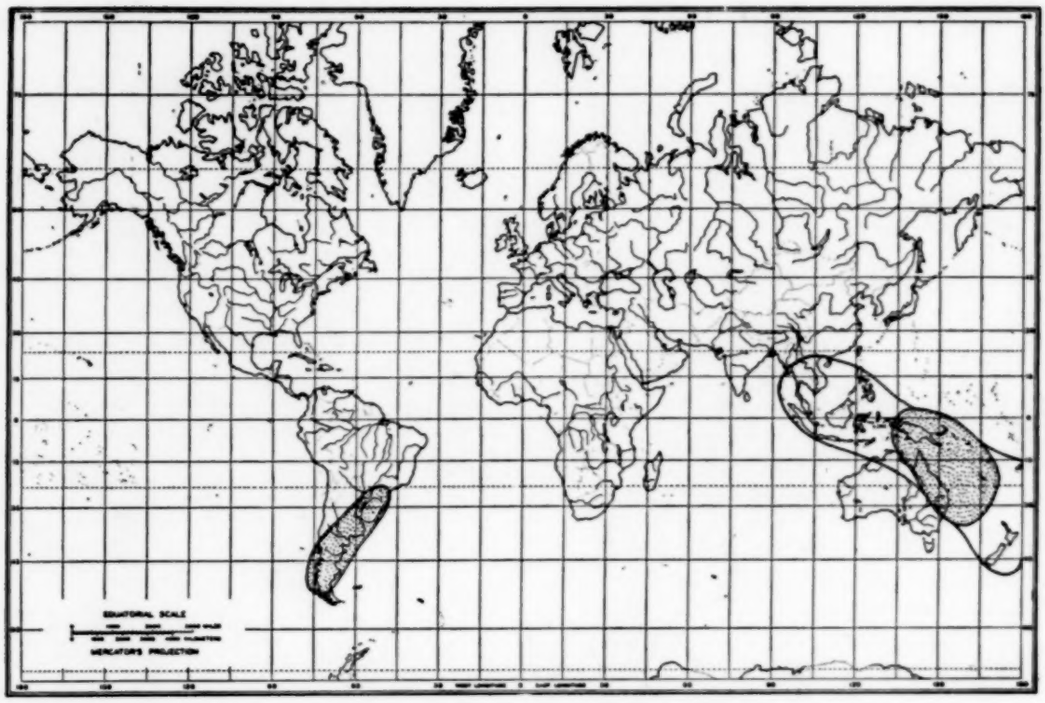
NORTHERN VERSUS SOUTHERN TYPES

The above table lists altogether fifty-four genera. Twenty-one are exclusively or primarily of the Southern Hemisphere, while thirty-three are exclusively of the Northern Hemisphere.[2]

[2] The genus *Libocedrus*, generally considered as of wide disjunct occurrences in both the northern and southern continents bordering the Pacific, is a composite group to be differentiated into three genera, *Libocedrus* and *Papuacedrus* in the south and *Heyderia* in the north (Li, 1953).

The clear differentiation of these genera into a northern and a southern group is a very conspicuous feature. Only in a few cases are occasional species extending to or beyond the tropics. In the northern genera, only *Pinus, Taxus* and *Juniperus* have a few species, which are more or less wide-spread in certain tropical regions, slightly transcend the equator. Among the southern genera, the northward transgression is more frequent and pronounced, which occurs in *Agathis, Dacrydium, Phyllocladus,* and *Podocarpus,* mostly toward southern Asia.

This northern and southern differentiation goes further beyond the generic rank. The families or subfamilies are almost equally well marked, in both the taxads and the conifers. In the Taxales, the



MAP 3. Generalized distribution of Araucariaceae.

LEGEND

○ AGATHIS

⊙ ARAUCARIA

Cephalotaxaceae and Taxaceae (frequently combined into one family), are all of the Northern Hemisphere, with the sole exception of *Austrotaxus* (Map 1),[3] while the Podocarpaceae are primarily of the Southern Hemisphere (Map 2). *Austrotaxus,* with one species in New Caledonia only, is little known and of doubtful taxonomic status (Florin, 1931). In general appearance and vegetative structures, it resembles closely *Podocarpus.* The reproductive structures suggest relationship with the Taxaceae. The genus may be a descendant from a certain ancestral type intermediate between the Taxacae and Podocarpaceae in characters. The geographical distribution and certain morphological characters indicate that the genus should perhaps more appropriately be referred to the Podocarpaceae.

In the Pinales, the Araucariaceae are primarily of the Southern Hemisphere (Map 3), while the related Taxodiaceae (Map 4) and Abietaceae (Pinaceae) (Map 5) are of the Northern Hemisphere. The only anomalous case is *Athrotaxis,* a genus of three species in Tasmania supposed to be related to *Cryptomeria* and *Taiwania.* The systematic position of *Athrotaxis* deserves further scrutiny. Florin (1940) is of the opinion that it is not of any direct relationship with the northern Taxodiaceae. The family Cupressaceae (Map 6), as here arranged, are clearly differentiated into two subfamilies, one of the north and the other exclusively of the south with the exception of the isolated *Tetraclinis* in northern Africa. Perhaps like other families, these subfamilies, other than general similarities in vegetative features, are actually of only remote relationships and they really deserve recognition in the familial rank (Li, 1953).

The present distribution of conifers and taxads indicates two separate areas

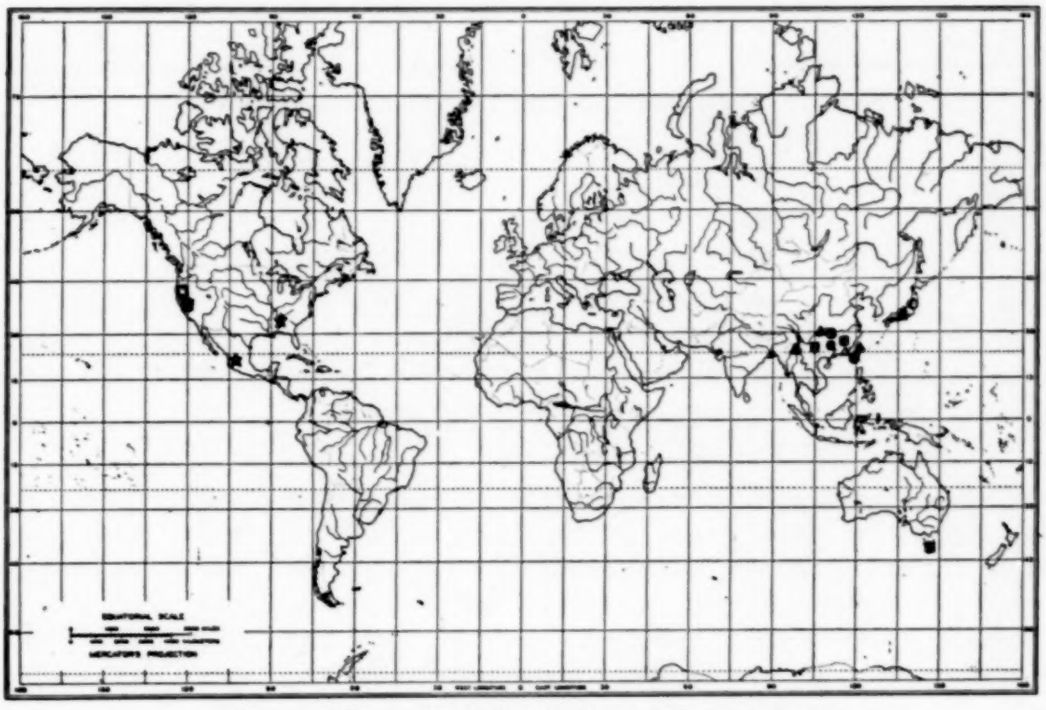
[3] All maps are based on Goode's Series of Base Maps No. 101M, published by the University of Chicago Press.

of development long independent of each other. Florin (1940) has proved that from fossil evidence the separation of the northern and southern types of the conifers and taxads has existed since the late Paleozoic.

Wide-spread versus Restricted or Disjunct Types

Among the northern genera, only six are of relatively wide ranges. These are *Juniperus, Cupressus, Abies, Picea, Larix* and *Pinus.* In the southern group, only one genus, *Podocarpus,* is wide-spread. As mentioned before, in all cases, the number of species in these genera is larger in regions bordering the Pacific than elsewhere. For instance, in *Pinus,* there are fifteen species in eastern Asia, seventeen in Pacific United States, and fourteen in Central America, all of greater number than any other areas where the genus is found (Map 5).

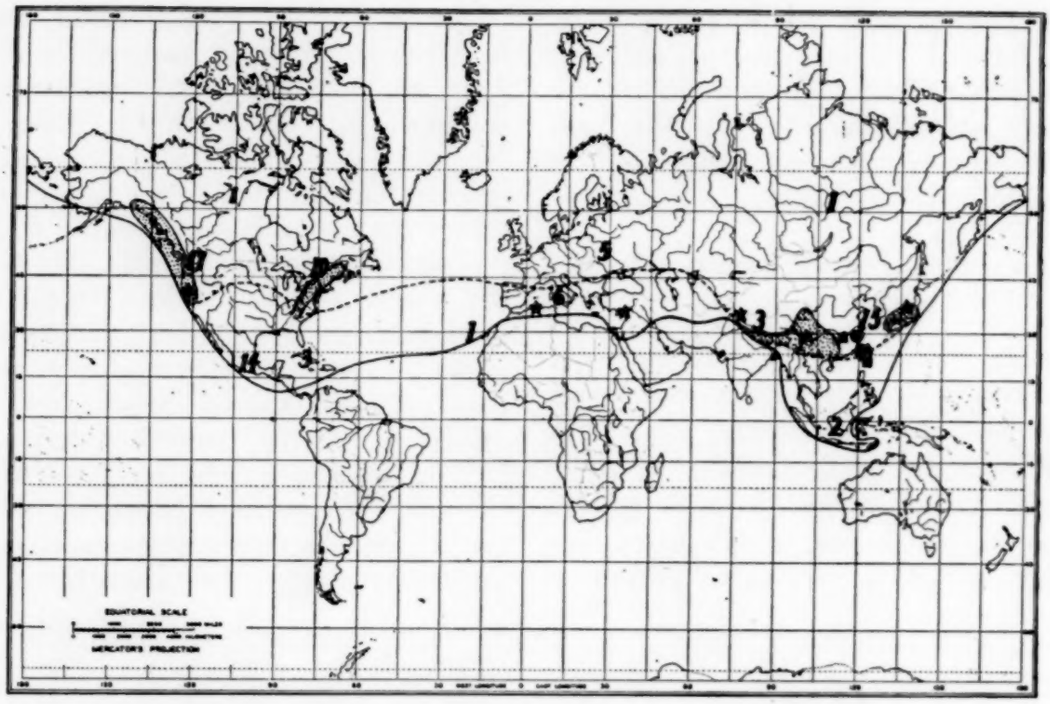
The polytypic genera with wide ranges are presumably genera in a recent cycle of speciation. The genera are all without doubt of very old origin, but some or most of the species apparently have arisen in relatively recent times. This can be attested by the fact that among the northern genera, it is species of these larger genera that occupy successfully and extensively the recently opened



Map 4. Generalized distribution of Taxodiaceae.

LEGEND

- Athrotaxis
- Cryptomeria
- Cunninghamia
- Glyptostrobus
- Metasequoia
- Sciadopitys
- Sequoia
- Sequoiadendron
- Taiwania
- Taxodium



MAP 5. Generalized distribution of Abietaceae (Pinaceae).

LEGEND

★ CEDRUS
■ KETELEERIA
● PSEUDOLARIX
▲ PSEUDOTSUGA
○ TSUGA
---- APPROXIMATE SOUTHERN LIMITS OF ABIES, LARIX, AND PICEA.
—— APPROXIMATE SOUTHERN LIMIT OF PINUS.
(FIGURES REFER TO NO. OF SPP. IN EACH REGION.)

northern lands after glaciation as well as the highest mountain tops of the temperate regions. On the other hand, the monotypic or oligotypic genera nearly always occupy very narrow and sometimes widely disjunct areas, indicating distinctly the relic nature of the species concerned. As is often said, the key of the past is to be supplied by the present, and as habitats are not readily revealed by paleobotanical records, a study of the habitats, especially of these relic genera of conifers and taxads, which are generically the majority of these plants, will serve to enlighten our understanding of the past history of these genera.

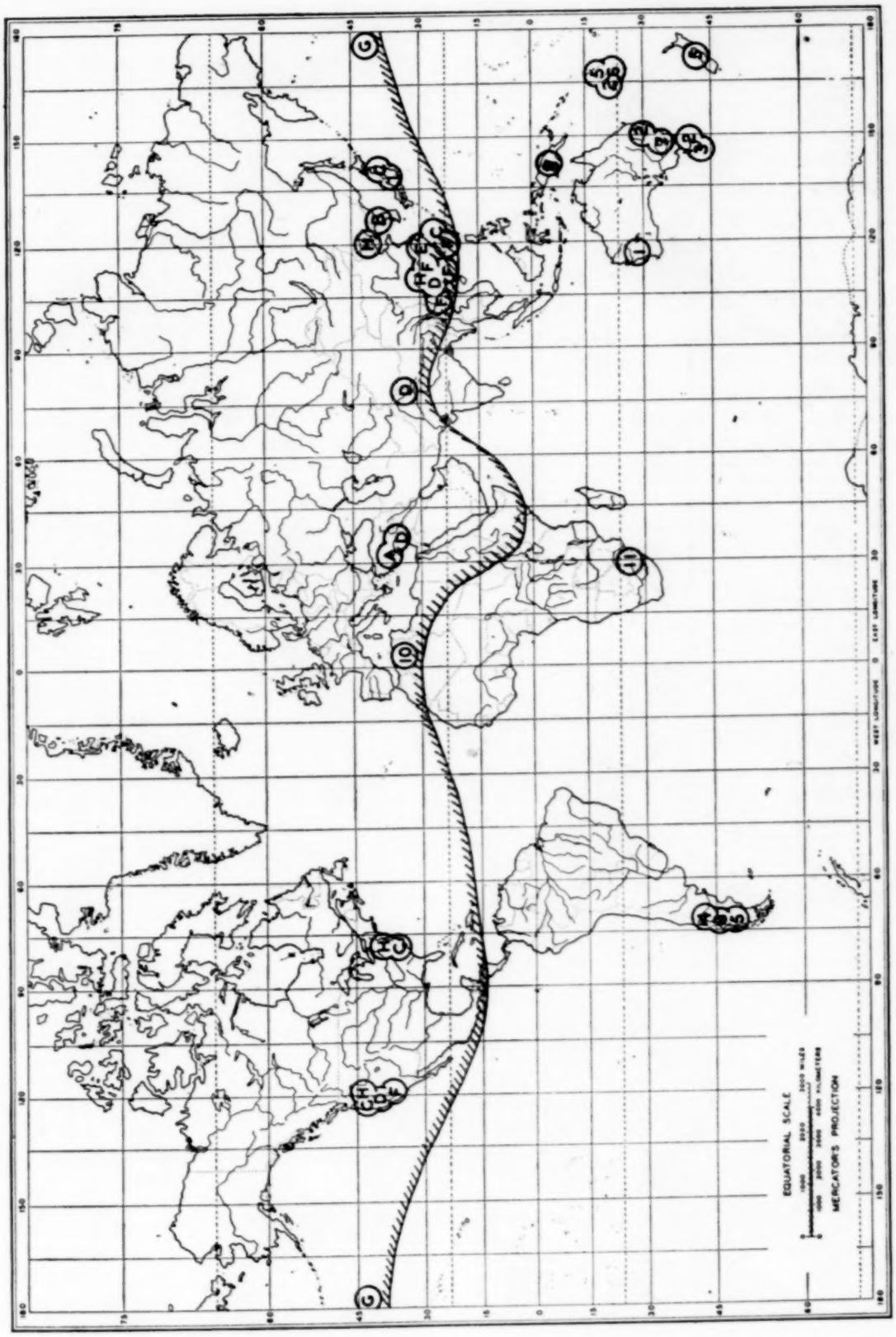
MAP 6. Generalized distribution of Cupressaceae

Subfamily Callitroideae

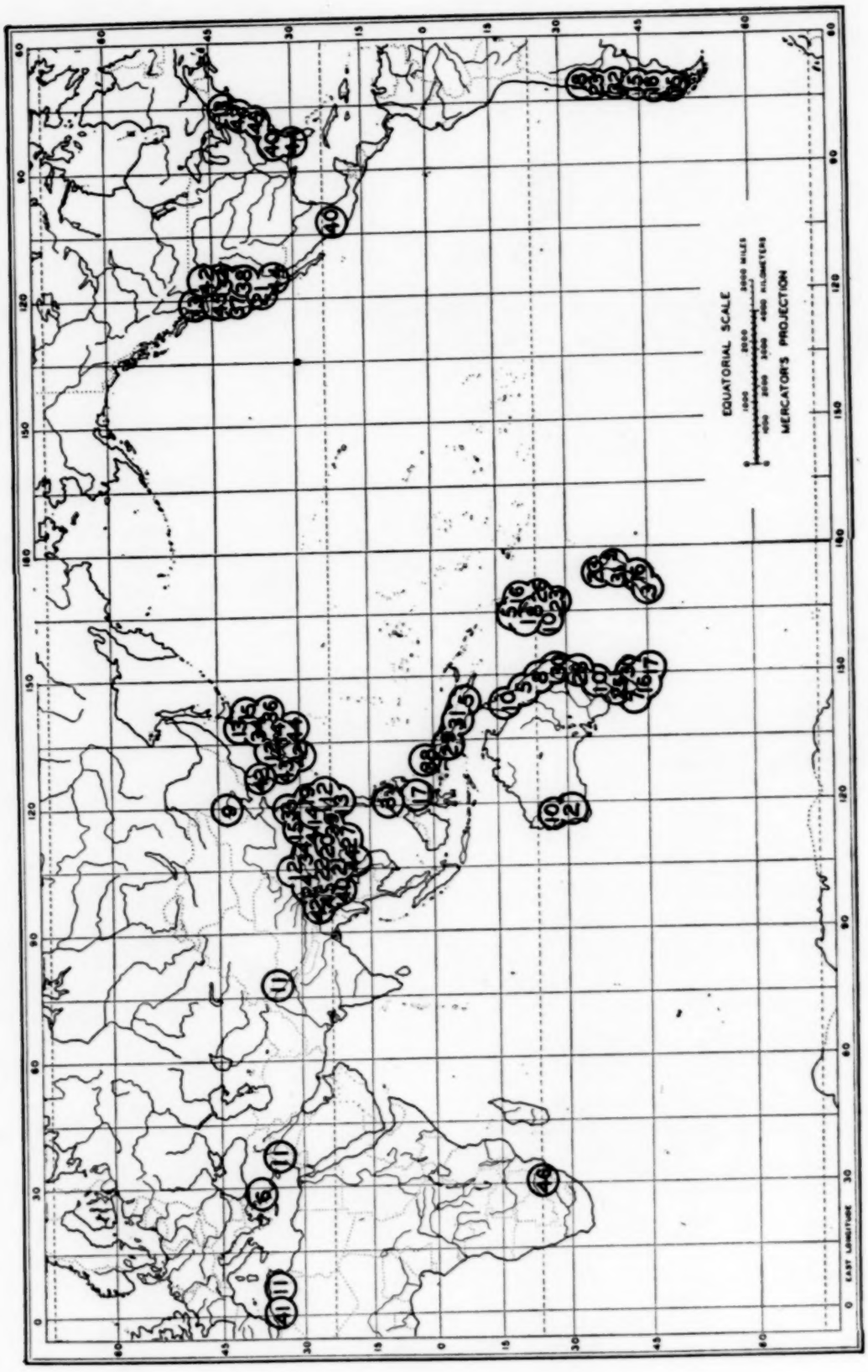
1 *Actinostrobus*
2 *Callitris*
3 *Diselma*
4 *Fitzroya*
5 *Libocedrus*
6 *Neocallitropsis*
7 *Octoclinis*
8 *Papuacedrus*
9 *Pilgerodendron*
10 *Tetraclinis*
11 *Widdringtonia*

Subfamily Cupressoideae

A *Arceuthos*
B *Biota*
C *Chamaecyparis*
D *Cupressus*
E *Fokienia*
F *Heyderia*
G *Juniperus*—approximate southern limit
H *Thuja*



MAP 6.



MAP 7.

Distribution and Habitats of the Wide-spread Polytypic Genera

The polytypic genera contain species of wide variations in both habitats and ranges of distribution. In every one of these genera, *Taxus, Podocarpus, Juniperus, Cupressus, Abies, Picea, Larix,* and *Pinus,* there are species of very wide ranges and others of very restricted distribution. Perhaps the species with the most extensive range is *Junpierus communis* L., a variable plant with diversified habitats, with ranges covering most of Europe, northern Asia, and most of North America. On the other hand, many insular and high montane species are of very restricted and local occurrence.

Among the species of these polytypic genera, there are ones that have preference of wet swampy habitats and others of relatively dry situations; but the majority are of mesophytic types, and these frequently attain their best development in relatively moist situations, as along banks of river or streams or close to bay and coasts.

The species that form extensive forests in the northern glaciated regions all belong to the few polytypic genera *Pinus, Picea, Abies,* and *Larix.* They extend to the very extent of the tree-line. The number of these species are very few, yet each often occupy great expanse of land, such as *Pinus sylvestris* L. and *P. cembra* L. in northern Europe and northern Asia.

Habitats of Restricted Genera

While a number of important forest trees of conifers and taxads have been extensively studied in various ecological aspects, it is surprising to note how little and inadequate our knowledge concerning many of the genera with restricted ranges, particularly those of the Southern Hemisphere. However, the general habitats of practically all genera are known and can be briefly summarized below. The genera are arranged in alphabetical order.

Acmopyle

There are 3 species in this genus, 2 in New Caledonia and 1 in Fiji, in moist forests on high mountains of about 900 meters in altitude.

Map 7. Generalized distribution of the genera of conifers and taxads (the following widespread genera are not included: *Taxus, Podocarpus, Juniperus, Cupressus, Abies, Picea, Larix, Pinus*).

1 *Acmopyle*
2 *Actinostrobus*
3 *Agathis*
4 *Amentotaxus*
5 *Araucaria*
6 *Arceuthos*
7 *Arthrotaxis*
8 *Austrotaxus*
9 *Biota*
10 *Callitris*
11 *Cedrus*
12 *Cephalotaxus*
13 *Chamaecyparis*
14 *Cryptomeria*
15 *Cunninghamia*
16 *Dacrydium*
17 *Diselma*
18 *Fitzroya*
19 *Fokienia*
20 *Glyptostrobus*
21 *Heyderia*
22 *Keteleeria*
23 *Libocedrus*
24 *Metasequoia*
25 *Microcachrys*
26 *Neocallitropsis*
27 *Nothotaxus*
28 *Octoclinis*
29 *Papuacedrus*
30 *Pherosphaera*
31 *Phyllocladus*
32 *Pilgerodendron*
33 *Pseudolarix*
34 *Pseudotsuga*
35 *Saxegothaea*
36 *Sciadopitys*
37 *Sequoia*
38 *Sequoiadendron*
39 *Taiwania*
40 *Taxodium*
41 *Tetraclinis*
42 *Thuja*
43 *Thujopsis*
44 *Torreya*
45 *Tsuga*
46 *Widdringtonia*

Actinostrobus

This genus is confined to western Australia. There are 2 species of densely branched shrubs growing along rivers and coastal regions.

Agathis

There are about 20 species of Kauri Pines in tropical and subtropical regions from the Malay Archipelago through the New Hibrides, Fiji, New Caledonia, Australia to New Zealand. The genus is essentially an island type. The species occur at medium to high altitudes. Three of the species occur in Australia, along coastal ranges.

Amentotaxus (Li, 1952)

There are 4 species of shrubs or small trees in the warmer parts of eastern Asia, mostly in subtropical regions. All species are of very restricted and rare occurrences, in moist damp broad-leaved forests in shady places, along edges of streams, or in wet rocky situations, at altitudes of 300–1500 meters.

Araucaria

There are about 20 species of Southern Pines. Two species occur in temperate South America, forming in places pure forests; 2 or 3 occur in Australia, 3 or 4 in New Guinea, about 8 in New Caledonia and the others in the islands of the southern Pacific. They are often trees of moist forests of islands or coastal ranges ascending up to 2000 meters in altitude in New Guinea.

Arceuthos

This is a monotypic genus of western Asia and southern Europe, the species often being included in *Juniperus*. It is a low tree occurring on mountains at about 550–1800 meters either as an undergrowth in Oak and Pine forests or in other places covering large stretches unmixed.

Athrotaxis

The Tasmania Cedar is a genus of Tasmania, of the temperate rain forests in mountain regions. There are 3 species, all of restricted occurrences, but locally dominant in sheltered gullies, ascending in places to the summit at 1300 meters. They grow especially near falls, around lakes and along rivers.

Austrotaxus

The only species of this genus is a tall tree of the moist forests of New Caledonia, occurring on steep slopes of 400–1000 meters in altitude in the northern part of the island.

Biota

The only species of this genus is sometimes included in *Thuja*. It occurs in northeastern Asia in Manchuria, northern China and Korea, and also in southwestern China in Yunnan. It is a very variable plant thriving in diverse soils and situations but it attains best development in moist situations.

Callitris

Callitris or Australian Cypress is a genus of about 20 species of trees and shrubs mostly in Australia with a few in Tasmania and New Caledonia. These species grow in woods or plains, near bays or rivers banks in the mountains.

Cedrus

The 4 species of Cedars occur in widely disjunct areas, 1 in the Atlas Mountains in northwestern Africa, 1 in Cyperus, 1 in Asia Minor, and 1 in the northwestern Himalayas. They ascend to 3200 meters in altitude, often forming extensive forests; and they grow in dry to moist situations but attain best development in the latter.

Cephalotaxus

There are 6 species of Plum-Yews in eastern Asia. All species prefer warm, moist and shady situations in the mountains varying from 330–2600 meters in altitude, among thickets and mixed forests.

Chamaecyparis

This genus of Cypress is confined to eastern Asia and North America. In Asia, there are 3 species in Formosa and Japan only. These are the tallest trees in eastern Asia. They grow at 600–2800 meters in altitude, forming pure stands. There are also 3 species in North America, 1 wide-spread in the Atlantic regions and 2 along the Pacific coasts. These species occur in the cold swamps of the coastal plain in the east and in ravines and moist hillsides of the coastal ranges in the west ranging from sea level up to 1000 meters in altitude.

Cryptomeria

Cryptomeria or Japanese Cedar occurs in China and Japan. The genus is generally considered monotypic, although the Chinese species is sometimes treated as a distinct variety or species. This tree is extensively planted, rarely seen in wild. It grows on foothills and mountain slopes up to 2000 meters and thrives in regions with mild equable temperature and abundant rainfall.

Cunninghamia

There are 2 species of Chinese Firs. One species is wide-spread in the temperate parts of the Chinese mainland, forming extensive forests, both planted and spontaneous, from sea level up to 2000 meters. The other species

occurs on the island of Formosa, at 1300–1900 meters in altitude, usually in *Chamaecyparis* forests.

Dacrydium

There are about 20 species of evergreen trees and shrubs, mostly of the Australian islands in New Zealand, Tasmania and New Caledonia, extending northward to the monsoon region from New Guinea, Philippines, Celebes, Borneo, Sumatra to the Malaya Peninsula, Annam, and Hainan. One species occurs in southern Chile at about 700 meters. The species are mostly plants of the mossy forests or river banks, occurring from sea level to high altitudes mostly between 600–2400 meters.

Dieselma

This is a monotypic genus of Tasmania. It is a shrub of very restricted habitats, occurring around lakes and falls in the mountain forests and shrubberies, ascending to about 1300 meters in elevation.

Fitzroya

There is only one species of Patagonia Cypress, a tall tree of southern Chile, abundant on coastal hills and becoming a bush at the snow line. It grows with *Pilgerodendron* in a region with most equable climate and abundant moisture.

Fokienia

This is a monotypic genus of southeastern China and northern Tonkin. The tree grows at altitudes of 200–600 meters in moist valleys and on slopes. The species forms extensive forests along the Yunnan-Indo-China border.

Glyptostrobus

This is a monotypic genus, a deciduous marshy tree, growing along water edges in southeastern China along the coastal regions. It also grows inland on hills and mountain slopes.

Heyderia (Li, 1953)

There are 3 species of Incense Cedars, 1 in Pacific North America and 2 in subtropical southeastern Asia. These species grow scattered in broad-leaved or coniferous forests, rarely forming pure stands, in ravines and mountain slopes at 300–2500 meters in altitude.

Keteleeria

There are about 4 species in the southern provinces of mainland China and the island of Formosa. These trees occur at medium altitudes from 300–1700 meters, either singly or in small groves. *Keteleeria davidiana,* the most wide-spread species, is especially abundant in regions with a relatively dry and hot climate, but this and the other species thrive also in warm moist climates.

Libocedrus (Li, 1953)

There are 6 species in the genus, widely scattered in regions bordering the southern Pacific, 1 in southern Chile, 2 in New Zealand, and 3 in New Caledonia. These are mountain trees growing on slopes and valleys varying from 950–1500 meters in altitude.

Metasequoia

The only living species of *Metasequoia* is a tall deciduous tree occurring in a small area in central China, in valley bottoms and slopes at altitudes of 400–2000 meters frequently on borders of flooded rice fields or streams and also extending up to ravines.

Michrocachrys

The only species, a low straggling shrub of the highlands of Tasmania, is a very rare tree occurring only on the highest elevations in the shelter of boulders.

Neocallitropsis

This is a monotypic genus of New Caledonia. It is a tree of the mountains as yet little known and collected.

Nothotaxus

This is a monotypic genus of eastern China. The species is a shrub or small tree growing in mountain forests at about 1000 meters in altitude, known as yet from a few collections in a small locality.

Octoclinis

This is a monotypic genus confined to the southeastern part of Australia. The species is often included in *Callitris.* It occurs in the mountains of coastal regions.

Papuacedrus (Li, 1953)

Among the conifers, this genus, a recent segregate from *Libocedrus,* is the only one that is confined to the tropics. There are 3 species in New Guinea and Moluccas, growing on ridges and in forests at 900–3000 meters in elevation.

Pherosphaera

There are 2 species of shrubs in this genus, of Tasmania and southeastern Australia. They grow on mountains at wettest situations at the base of waterfalls and fringing lakes and water courses.

Phyllocladus

There are 6 species of Celery-topped Pines, 3 in New Zealand, 2 in Tasmania, 1 in New Guinea, and 1 in the Malayan region. They grow from sea level to high mountains in New Zealand. In tropical regions the species occur only on high mountains at 1200–3000 meters in altitude. The trees mostly grow in damp mossy forests.

Pilgerodendron

This is a monotypic genus of southern Chile, growing at altitudes of 600–1000 meters along the western slopes of the Andes. It is a tall tree, often associated with *Fitzroya*. It occurs in a region of one of the most equable climate and one of the most humid of the world.

Pseudolarix

The Golden Larch is a monotypic genus of the mountains of eastern China at altitudes of about 300–1000 meters. This is a deciduous tree that thrives in a temperate climate with abundant moisture.

Pseudotsuga

There are 6 species in the genus, 4 in eastern Asia and 2 in Pacific North America. The Asiatic species are generally of restricted ranges and rare occurrences. They are mountain trees occurring at 700–3300 meters in elevation. The American species are more widespread and abundant, growing on lowland plains and in valleys in the north in British Columbia and up to 3500 meters in elevation in the south in northern Mexico.

Saxegothaea

The Prince Albert Yew is a monotypic genus of southern Chile. It occurs on mountains at about 150–200 meters in altitude in damp forests formed together with other conifers.

Sciadopitys

The Umbrella Pine is a monotypic genus peculiar to Japan. It is a tree of the mountains at 1300–2000 meters in elevation, mixed frequently with *Chamaecyparis*. Perhaps all plants are planted, and these grow in retentive moist soil.

Sequoia

The only species, the well known Redwood, occupies a narrow belt of the littoral region of Pacific North America, forming pure forests in northern California, on the sides of ravines and the banks of streams. This is a lover of moisture and a characteristic tree of the coastal fog belt.

Sequoiadendron

The only species, the Big Tree, the largest of all trees, has a restricted range in California, confined to a narrow belt along the western slopes of the Sierra Nevada, at 1650–2650 meters, an elevation which is precipitated for nearly six months of the year.

Taiwania

Taiwania is a genus of the warmer parts of eastern Asia. Two species are known, one in Formosa, scattered in *Chamaecyparis* forests at 1800–2600 meters in altitude, and another in southern Yunnan and northern Burma and also in western Hupeh in mountain valleys at about 900 meters in altitude. The Formosan species grows in deciduous broad-leaved forests, and the latter species in evergreen broad-leaved forests.

Taxodium

There are 3 species of Deciduous Cypresses, 2 in southeastern United States, semi-aquatic trees growing in or near water or in low swamps, and a third on tablelands of 1400–2300 meters in elevation from Texas to Mexico.

Tetraclinis

The Alerce, the only species of the genus, occurs in northern Africa, in Morocco, Algeria, and Tunisia. It forms forests with *Juniperus, Taxus,* etc. in the Atlas Mountains.

Thuja

There are 5 species of Arbor Vitae. Two species occur in North America, one along the Atlantic coast common in great area of swamp land, and the other along the Pacific coast forming extensive forests especially along the Columbia River valley. In Asia, 1 species occurs in Japan, 1 in Korea, and a third in western China, all of very restricted ranges and more or less rare occurrences. They generally grow in mountains at 700–1800 meters in altitude, prefering shady moist situations.

Thujopsis

This is a monotypic genus of Japan. In the northern part of its range it is a tall tree forming forests on plains and on mountains. The southern variety is a small tree or straggling shrub, growing at high altitudes up to 2300 meters, forming undergrowth in the shade of dense coniferous forests.

Torreya

There are 6 species in discontinuous areas in warm temperate and subtropical Asia and North America. Two species occur in North America, one of restricted area in Florida on the bluffs of the Appalachia River and the

other widely distributed in California, inhabiting borders of mountain streams at 1000–1650 meters in elevation. In Asia, 1 species occurs in Japan and 3 in China, growing along banks of rivers, edges of streams or in ravines at 100–1600 meters in altitude.

Tsuga

There are about 10 species of Hemlocks, all of the high mountains of the temperate regions in eastern Asia from the Himalayas through China to Japan and in North America in both the east and the west. The altitudinal range is from 400–3300 meters. The trees sometimes form pure stands and often thrive best along banks of rivers.

Widdringtonia

Widdringtonia or Cypress Pine is a genus of southern and southeastern tropical Africa. There are 5 species generally of damp mountain forests up to 2600 meters high in tropical regions.

Summarizing the above brief account, it can be seen that a small group of genera are those with a decided preference of very wet situations, such as swamps, river banks, etc. In this group are four genera, including the well known *Taxodium* of North America, and *Glyptostrobus* of southern China, both of swamps and water edges. *Metasequoia* of central China frequently grows along flooded fields. Both *Taxodium* and *Glyptostrobus* also grow on higher lands in the more northern parts, attesting to their wide amplitude of ecological tolerances. In the Southern Hemisphere, *Pherosphaera* of Tasmania and southeastern Australia grows on mountains at wettest situations fringing lakes and water courses and at base of waterfalls.

Most of the other genera are those with a mesophytic habitat but show decided preference of damp situations. They either require sufficient moisture in the ground or also a damp atmosphere. While some of the species may grow in a wide range of situations, most of them are of the moist forests of the temperate or subtropical regions, and they almost always attain their best development where ample amount of moisture is present, such as along rivers, streams, etc. In nearly all cases, the species thrive best in hilly districts and on mountain slopes. This group contains the majority of these genera in both the Northern and Southern Hemispheres.

A very small number of genera thrive in mesophytic habitats frequently in drier situations. But they generally also prefer retentive soil and develop best in situations where moisture is relatively abundant. These genera are *Arceuthos, Biota, Keteleeria,* and *Microcachrys.*

Correlation of Habitats and Distribution

The notable features of distribution in the majority of these conifer and taxad genera, aside from their concentration along the Pacific basin are the following:

(1) Most of the genera grow in mountainous situations;

(2) Most of these genera occur in warm temperate regions, especially in areas with equable climate;

(3) Most of these genera show definite preference to moist situations.

A region that combines all these various conditions is most frequently of coastal location and this is exactly where the majority of these genera occur.

The great concentration of these genera along the Pacific basin and their near absence along the coastal areas of the Atlantic and Indian Oceans are in striking contrast (Map 7). We may safely infer that a region with even temperature and abundant moisture will maintain the same habitat if the geography of the region did not alter much through the ages. The present distribution of the conifers and taxads therefore indicates that the Pacific basin is not only much older than the other ocean basins, but that the coasts have remained as such in a more or less similar condition ever since the Mesozoic, when the conifers and taxads were at the zenith in their development. On the other hand, the Atlantic coasts cannot be similarly dated in so far as conifers and taxads are concerned.

Lands of Conifers and Taxads

The regions where the relic genera of conifers and taxads are especially abundant are relatively few in number but of very great floristic interest. These are southern Chile, Tasmania, New Caledonia, Formosa, southern-eastern-central China, Japan and the Pacific United States. Of lesser importance are New Zealand, eastern Australia, and New Guinea. All these regions border the Pacific (Map 6).

In southern Chile, besides *Podocarpus, Dacrydium,* and *Libocedrus,* there occur the monotypic endemic genera *Fitzroya, Pilgerodendron,* and *Saxegothaea.* These conifers and taxads form extensive forests along the Pacific slopes of the Andes.

Tasmania is well known as a land of ancient biota. It has nine genera of conifers and taxads including the more widespread *Callitris, Dacrydium, Phyllocladus,* and *Podocarpus* and the more restricted *Pherosphaera,* which has another species in southeastern Australia, and *Athrotaxis, Diselma,* and *Microcachrys,* all endemic.

In New Caledonia, another ancient land with a most extraordinary and rich flora, there are nine genera and forty species of conifers and taxads. The genera *Austrotaxus* and *Neocallitropsis* are endemic and the genus *Acmopyle* is found in New Caledonia and Fiji only. New Caledonia is the center of development of the genus *Araucaria,* where seven out of the total twelve species occur.

The island of Formosa, lying along the Tropic of Cancer, has a comparable position along the eastern border of the Pacific in the Northern Hemisphere as New Caledonia is in the Southern. This small island has the remarkable number of fiften genera and twenty-seven species of conifers and taxads. The genera *Amentotaxus, Keteleeria, Cunninghamia,* and *Taiwania* occur disjunctly on the Chinese mainland only.

In southern-eastern-central China, there are no less than twenty-four genera and about 110 species of conifers and taxads. Besides the four genera shared with Formosa, there are five additional endemic genera, *Pseudolarix, Nothotaxus, Fokienia, Glyptostrobus,* and *Metasequoia. Biota* is also found in Korea, *Cryptomeria* in Japan, and *Heyderia* in Formosa and Pacific North America. In addition, there is *Gingko,* an even more ancient type than most genera of conifers and taxads, preserved in cultivation or in a semi-wild state in China only.

In Japan, excluding Sakhalin and Hokkaido in the north and the Liukius in the south, there occur sixteen genera and thirty-nine species of conifers and taxads. The genera *Thujopsis* and *Sciadopitys* are endemic.

In Pacific North America, there occur sixteen genera (including *Taxodium* of Mexico) and about sixty species of conifers and taxads. The genera *Sequoia* and *Sequoiadendron* are endemic.

All these lands mentioned are warm temperate or nearly subtropical in climate, along or close to the Pacific coasts. The seasonal fluctuation in temperate is much narrower in these areas than in most other places. In all regions, rainfall is abundant. It is suggested that only through long maintenance of such a situation that these relic genera of conifers and taxads are able to survive up to the present.

The significance of a Pacific location is readily seen when, for instance, the island of Formosa is compared with the slightly larger island of Cuba, which has a more or less similar latitudinal location but along the Atlantic instead of the Pacific. While Formosa has fifteen genera and twenty-seven species, Cuba has only three genera and ten species, and these genera, *Pinus, Juniperus,* and *Podocarpus* are all very wide-spread. Similarly the island of New Caledonia can be compared with the much larger island of Madagascar, situated on about the same latitudes but along the Indian instead of the Pacific Ocean. Both have

an extraordinarily rich flora with exceedingly high incidence of endemism. The distinctly rich flora of conifers and taxads of New Caledonia, however, is in striking contrast with the near absence of conifers and taxads in Madagascar. Another comparison can be made between the Pacific and Atlantic coasts of North America. Along the Atlantic coast of North America, where the largest concentration of conifers and taxads outside the Pacific area is found, there are only eleven genera and about twenty-seven species, much less than the same flora along the Pacific coast.

In this connection it is worthy to note that the ten known genera of Angiosperms with the archaic vessel-less type of wood like that in conifers and taxads, are all found in these same areas as noted above; two are in New Zealand only, three in eastern Asia, and five in the New Caledonia area with three of them endemic only to the island.

Conclusions

It is conceivable that the climate during the Mesozoic, when conifers and taxads were best developed, was as variable as it is now, as there must have been differences due to altitudes and latitudes then as now. Apparently the different habitats were all populated by some types of conifers and taxads. From the existing forms, we may, however, infer that the best suitable habitat for most of the conifers and taxads then was also a moist mesophytic one. At least more genera of this habitat are preserved in the living state up to the present.

It is also conceivable that extreme dry and wet habitats have little opportunity of maintaining the same state for very long periods in geological times. A moist mesophytic habitat, on the other hand, has the possibility of remaining as such along the coast of ocean. This will be possible either by means of the geological stability of the area or more plausibly by the mountainous topography of the region as plants may ascend or descend when the region is submerged at different levels. The chance of preservation of the same habitat therefore is much greater than in inland areas.

The great concentration of the relic genera of conifers and taxads, practically all in favor of a moist mesophytic habitat, along the present rim of the Pacific Ocean, which is largely mountainous, but not along other ocean borders, is therefore not a mere coincidence. Also very significant is the fact that the northern and southern types are very distinctly separated in their distribution.

The traditional view of the northern origin of all conifers is apparently in need of revision. As far as the southern conifers are concerned, Florin (1940) is of the opinion that the distribution data indicate land connections, or at least much smaller distances than now, between Antarctica and the adjacent ends of South America, Australia, New Zealand, and South Africa.

The disjunct distribution of conifers is frequently cited as evidence toward the Wegner's theory of continental drift. Koch (1924) believes that the conifers were originated in the tropical flora of the Carboniferous and soon differentiated into a northern and a southern group as the result of the changing climatic conditions. Studt (1926) is of the opinion that all conifers originated in the north temperate zone, from whence they spread northward as well as southward to the Southern Hemisphere. He believes that the differentiation into a northern and a southern group did not occur until the late Mesozoic. Florin (1940), however, refutes this idea and instead proves convincingly the very early differentiation of the northern and southern types.

The very clear distinction of northern and southern types seems to be in favor of the supposition of two early land masses of Du Toit (1937). The disjunct distribution of some of the southern genera on both sides of the Pacific, like *Dacrydium, Libocedrus, Araucaria,* and *Podocarpus,* is considered by some recent

botanists, such as Campbell (1943) and Buchholz (1948), as strong evidence toward the theory of continental drift as modified by Du Toit.

Modified forms of the drift theory, especially that of Du Toit, are favored by many paleobotanists and plant geographers (for summaries, see Just, 1947, 1952, Good, 1950, 1951). It is the thesis of Du Toit, modifying the earlier views of Wegener and others, that there were two ancient land masses, Gondwana in the south and Laurasia in the north, separated by the Tethys Sea. When the present ranges of these numerous relic genera of conifers and taxads are superimposed on the outline of these early continents, in all cases they lie along the marginal areas. The few genera of extra-Pacific areas are thus similarly located, *Widdringtonia* of southern Africa, on the southern margin of Gondwana, and *Tetraclinis, Arceuthos* and *Cedrus,* on the border of the Tethys Sea.

The association of all the relic genera of conifers and taxads with ancient coast lines seems to be distinctly indicated. These genera survive in their present locations evidently because of a more stable habitat. That these habitats remain long unchanged or little changed along the Pacific border and the ancient Sea of Tethys show that these were along the marginal regions or remnants of margins of land masses, where climatic fluctuations were least marked throughout the long histories of the continents as well as of these plants.

Could it then be that the obvious relationship of these genera with the Pacific basin should actually be interpreted as the preservation of these ancient plants along more or less premanent margins of ancient land masses because of the long stability of their required habitats? As an evidence toward the continental drift theory, this is indeed indirect, but coordinated with the distributional pattern of those disjunct genera of conifers and taxads which favors this theory as a working hypothesis in plant geography, this phenomenon is worthy of note.

Summary

The present distribution of the conifers and taxads indicates a clear differentiation into a northern and a southern group. This separation has been shown by paleobotanical records to have existed since the late Paleozoic. Nearly all of the large number of monotypic and oligotypic genera, mostly of restricted or disjunct distribution, thrive best in a damp mesophytic habitat. These genera are now practically all confined to lands bordering the Pacific Ocean. It is assumed that these favorable habitats remain long unchanged or little changed because of their location along the marginal areas of two ancient land masses. The disjunct distribution of many of these relic genera is considered by some botanists as evidence toward the theory of continental drift as modified by Du Toit. The more or less restricted and uniform habitats of these genera seem to corroborate such an assumption.

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ORIGIN OF THE VARIATION IN THE IMPORTED FIRE ANT

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Two years ago (Wilson, 1951) I described the rapid evolutionary change which appears to have taken place during the past twenty-five years in an introduced population of the fire ant *Solenopsis saevissima* (F. Smith) in the southern United States. This population is thought to have originated at the port of Mobile, Alabama, sometime around 1918. For about ten years it remained both genetically homogeneous, corresponding to the dark southern race *richteri* Forel of the South American parental population, and relatively unsuccessful in its new surroundings. In the period following 1930 a smaller reddish form rose to abundance, interbred extensively with the original dark form, and apparently precipitated the species' explosive increase to pest proportions. By 1949 the reddish form had largely replaced the dark form, which had become limited principally to the southern strip of the main population and part of its eastern and western periphery and to two outlying, isolated populations in Mississippi.

Recent revisionary work on the *Solenopsis saevissima* complex in South America (Wilson, 1952) has shed much light on the probable origin of the two extreme forms of the variation within the Gulf States population. It is now clear that forms morphologically identical with the "light" and "intermediate" phases of the Gulf States population occur commonly in the zone of intergradation between *Solenopsis s. saevissima* and *S. s. richteri* in South America. These are among the several variants, some of which show divergent characters of their own, which characterize the zone of intergradation. So far as is known, none of these variants possesses a range exclusive enough to deserve formal recognition as a subspecies; moreover, all show color characters intermediate between those which mark the terminal subspecies. The blend zone is exceptionally broad and with a decided clinal structure relative to color and size, although there are numerous examples of local reversals in the clinal trend.

As shown in the accompanying figure, the light and intermediate phases are concentrated in the central and western parts of the blend zone, occupying an area which falls mostly within the subtropical savanna of the Gran Chaco. On the other hand, the race *richteri*, including the dark phases of the Gulf States population, is excluded to the south and east of the Chaco, the greater part of its range lying within the more arid Pampas. (The light phases referred to here include the "light red" and "dark red" phases of the 1951 study, the intermediate phases include the "light intermediate" and "intermediate" phases, and the dark phases include the "dark intermediate" and "extreme dark" phases.)

The simplest explanation of the origin of the variation in the introduced population would seem to be a double introduction into Mobile, the first of the race *richteri* around 1918, and the second of the light phases (or possibly the intermediates, from which the light phases segregated) around 1930 or later. These supplied the two extremes of the variation between which the total variation is at present deployed along an almost straight gradient line of color and size. The warm temperate Gulf States environment has given the light phases an

FIG. 1. Distribution of the major variants of *Solenopsis saevissima* in South America. The light phases are indicated by arrows. The size of the symbol indicates the number of nest series examined from that locality, this number ranging from one to twenty. Several records of the typical *saevissima* and eastern part of the blend zone are from Creighton (1930); all other records are either from Wilson (1952) or hitherto unpublished.

advantage over both the dark phases and the intermediates.

A great deal yet needs to be learned about the variation within this introduced population. An outstanding problem is why the variability of individual colonies is so restricted despite the very heterogeneous nature of many local subpopulations (Wilson, 1951). Another problem is presented by the distributional data from the South American population: if the race *richteri* is indeed adapted to drier conditions, it seems contradictory that in the face of competition from the light phases it should survive longest in low, swampy areas. Perhaps we shall find that temperature is actually the principal physical selective agent involved.

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NOTES AND COMMENTS

PRICKING A BUBBLE

A critical review of N. P. Dubinin's 1948 paper on Experimental investigation of the integration of hereditary systems in the processes of evolution of populations [1]

RICHARD B. GOLDSCHMIDT

"In the face of a fact there is only one possible course of action for the scientist, namely acceptance, no matter how much the fact may be at variance with his anticipations and no matter what havoc it may wreak on his carefully thought-out theories." P. W. Bridgman

Professor Michael Lerner kindly made available to others and myself a translation of Dubinin's most recent paper (1948) on a topic of population genetics. As Dubinin is one of the pioneers and most prominent contributors in this field, many geneticists will be interested in what may have been his last contribution to genetics. As some of the features of the paper were supposed to have a close resemblance to results of our own work (1951) on podoptera (an apparent fact to which Professor Lerner drew my attention), I was glad to have an opportunity to study it in Lerner's translation. I must confess that I have rarely received a similar shock as when the study of the paper revealed that the conclusions of an extreme hyperselectionist type were based upon a complete misinterpretation of rather simple facts. It would not be necessary to expose the errors in detail, were it not for the fact that the critical observer finds a tendency towards an extreme conformity in interpretation in much of the work in population genetics. This uncritical conformity in the conclusions from facts which offer themselves to different interpretations appears to this onlooker an unhealthy development. Therefore the exposition of one rather bad case, committed by a leader in his field, may have some salutary effect. Actually Dubinin's paper is one of the worst cases of self-deception I have ever observed. Therefore I am willing to acept the odium of this probably unwelcome critique in the hope of accomplishing some good.

There is a special reason why I feel called upon to perform the operation. Dubinin's paper deals with some second chromosome mutants of *Drosophila*. The reader who is unacquainted with this material will accept the author's statements at their face value, assuming their inherent correctness. When he meets them with conclusions which have his sympathy he is led to believe that the facts bear out the conclusions and will not take the trouble to scrutinize the facts. I might have fallen myself into this psychological trap were it not for the fact that I have a special knowledge of the phenotypes and gentoypes involved. My own work (Goldschmidt, 1945, read p. 405 ff.) was done for a completely different purpose and it is hidden in a paper on spontaneous mutation. But it gave me a good knowledge of the material, the mutants in the second chromosome producing extra veins; their genetics, phenotypic expression, dominance, modificability by environment and genetic modifiers, multiple alleles, deficiency effects etc. were studied. In one table (79) the phenotypes of 54 genotypes were described and a photographic plate illustrates some of the facts. It is on the basis of this experience that I looked into the details of Dubinin's paper when I found strange and conflicting statements.

Dubinin's work deals with the occurrence of extra veins on the wing of *Drosophila* and this feature is found so widely spread in natural populations that he considers it of an obligate nature (in the sense of obligate for the existence of the species) and describes it as "aberrant polymorphism." In the paper he states at once the dogma (also contained in the title of the paper), upon which the work is based (p. 5 Ms.): [2] "The special genetic analysis carried out on a whole number of populations has indicated that populations are saturated with elements of the hereditary polygenic system which in such a small degree expresses itself in the appearance of extra veinlets on the wings of individuals in nature. These facts threw new light on the whole problem. It

[1] Dubinin, N. P. 1948. Experimental investigation of the integration of hereditary systems in the processes of evolution of populations. (in Russian) Zhurn. Obshch. Biol. **9**: 203–244.

[2] Page quotations refer to manuscripts of Lerner's translation. Ozalid copies of this translation can be purchased for $4.00 each on application to the Librarian, University of California, Los Angeles 24, California. A limited number of copies are available for inter-Library loans.

became clear that there appeared before us a heterogenic and at the same time a stable heheditary system of importance to the whole species. It is evident that only natural selection can be the cause of such stability of heterogenic heredity in populations." Further, "When we turn to the study of polymorphism in nature with respect to extra venation, a picture of qualitative variability, which seemingly was based entirely on the action of separate independent mutations, was again revealed. However, a detailed genetic analysis of this trait demonstrated that the basis of polymorphism for extra venation lies in a complex polygenic system. The investigation also revealed that this system has an adaptive significance and that natural selection has created in this case a particular intra-chromosomal organization." It will be shown that the experimental facts presented by this author do not bear out a single one of these and later more extreme conclusions, except the fact that the mutants involved are surprisingly frequent in natural populations. It will be shown that all the authors did was to extract two well known second chromosome mutants from the many populations in which they were found floating.

Here are the facts and interpretations in the order of their presentations along with some comments though it will be seen later that the genetic analysis presented towards the end of the paper nullifies most of the claims made in the earlier parts.

1. In the populations studied three types of extra venation are found, a free veinlet in the posterior cell, a veinlet attached to the posterior cross vein, and a little network of veins all over the wing or localized at the place of the free vein. (I add already here—consult table 79, and also tables 95–97, of my paper of 1945 as well as the photos that these phenotypes are known as the homozygous or heterozygous expression of the mutants net, blistered, balloon, plexus, the Plexate deficiencies and the loss of the entire right end of the chromosome as analyzed in detail in my paper. It will be seen later that two of them, net and blistered, were actually involved.)

2. The average number of these aberrant individuals in the different populations is only .29%. The third variant (network) is still rarer and found only in populations with high incidence of abnormals. (It will be shown that this is the mutant net which in the heterozygous condition is very similar or identical to blistered which is also present. High incidence of abnormals thus means the presence of heterozygous net, more rarely homozygous net and homo- and heterozygous blistered.)

3. The first two venation types seem to be related and, in addition, are always present in heterozygotes obtained from network found in nature; but the same phenotypes taken from nature did not segregate network. (This and many following data would appear less mysterious if it were simply stated, as it turns out later, that two ordinary mutants are present in the same chromosome. They are blistered (bs) and more rarely net (net). The first can have in the homo- or heterozygous condition the types of veinlet. The second may produce the same phenotype when heterozygous (see Goldschmidt, 1945). The phenotypic overlap of two different standard mutants is responsible for the gross errors in this paper. Net did not segregate directly from specimens in nature because it is very rare (see no. 2) and therefore most matings involved the phenotypically identical blistered or blistered × net/+. If he had tested many more pairs he would also have found the cross net/+ × net/+.) At this point a most amazing fact must be recorded. The author (see p. 14) studied the offspring of two "network" females from nature, i.e., the mutant net as turns out later. All offspring showed some extravenation (which is known for heterozygous net) from which the author concludes that network is a "genotypicaly homozygous" form. One should expect now a statement about F_2 (which, of course would have segregated 1/4 net). Not a word about this and nowhere else in the paper such a F_2 is mentioned. But it is stated that many matings of the extravein types *from nature* did not segregate network (of course because they did not contain net but blistered). Did Dubinin really miss to make the F_2 which would have exploded his entire story from the beginning? And why did he not repeat it after he found out, subsequently, that he had both net and blistered in his material?

4. The expression of these traits is different in the two sexes. "Some form of natural selection has regulated this difference." (I had pointed out (1945) that in all the dozens of genotypes studied the male phenotype is always one class below the female in plexation, and many of these mutants and combinations are laboratory products upon which no selection had acted. The cause is clearly one of embryonic determination, different speeds and rhythms of development, etc. In Dubinin's later discussion much is made of this sexual difference, and wild theories are advocated to explain what obviously is a frequently found simple fact of development. However, this is not a major point.)

5. In the females (not the males) the extra venation has a seasonal cycle, increasing with the maximum population size in summer. This is again ascribed to selection.

6. The characters veinlet and free vein are not completely penetrant; therefore some phenotypically normal flies are genetically abnormal, and this raises the percentage of the mutants in the populations above the level of .29% found.

7. A check of many normal not virgin females from nature showed that lines with extra venation could be derived from normal females in 67.9%. Among the other 32.1% heterozygotes must have been present because extra veins appeared after crossing of some of these lines, so that the author believes that the character is omnipresent. This is considered "the amazing fact of tremendous saturation of populations with hereditary elements for extra venation." The calculation is not convincing. The prefertilized females may have mated with many males only one of which may have carried the semi-dominant character. As mass selection was practiced as well as brother sister matings (see p. 21) the (subsequent) isolation of extra veins does not at all measure the genetic constitution of the original prefertilized females. In addition no genetic checks were made at this stage of the work. Later the mutants net and blistered were isolated. But among the thousands of not tested flies with extra veins present in these experiments may have been any of the other mutants within and without the second chromosome which produce extra venation.[3] This makes this experiment a body of unanalyzed data. If it were assumed that the *phenotype* extra veins is always combined with a beneficial physiological action, whatever be the genotypic causation, these experiments would reveal an interesting situation. A genetic analysis could show the postulated polygenic system. Actually when it was made, nothing of the kind was found, as will be shown soon.

It is nevertheless true that the presence of mutants producing extra venation is still an unexpectedly high one. This is, I think, the *only result* of the work. It would have been worthwhile to analyze this in ecological experiments, e.g., following Dobzhansky's procedure. But the author prefers to state ex cathedra that he found an obligate, adaptive polymorphism and he invents, without a genetic test, a polygenic system as the basic agent. Later on, when the genetic test was made this genetic system disintegrated, as will be seen below.

8. A similar situation was found in other populations from widely different climatic zones. "This indicates the existence of a hereditary system which is obligate for the species as a whole."

9. The same phenotypes are also found in a similar way in populations of other *Drosophila* species. Therefore "the heterogenic hereditary system" has a "significance for the genus as a whole." (No genetic check of these phenotypes was made, and the phenotypes may have been based upon completely different mutants and/or environmental actions. It is known that plexation is easily produced as a phenocopy. Here is a problem, but no attempt at experimental attack.)

10. By selection and inbreeding, penetrance and expressivity of the extra veins can be increased. In many cases the highest type network is obtained. (This selection work was done, as will appear later, without regard for the fact that he selected simultaneously and without discrimination a) for homozygosity of the simple Mendelian mutant net, b) for homozygosity of the mutant blistered (or balloon or both?) in the same chromosome, c) for the combined presence of both (or more) mutants in different homozygous and heterozygous combinations, d) for dominance enhancers, e) for multiple alleles of the loci involved (see again my paper for bs-alleles), and f) for enhancers of expressivity. As can be seen from my work quoted above, expressivity is easily enhanced, e.g., by third chromosome inversions, and is easily lowered, e.g., by the presence of the mutant dumpy. How can any conclusions be based upon such indiscriminate and uncritical procedure? However, this is still harmless compared with what follows now.)

11. Only at this stage of the presentation (p. 35) does the author come to what should have been the beginning of the story, "the analysis of such lines which have transformed themselves into lines with the extreme form in the shape of the network trait." (Notice the expression "have transformed themselves" for a simple extraction of a well known 100% penetrant and expressive mutant.) Network (actually the standard mutant net, as is subsequently admitted) is semi-dominant, the heterozygotes showing a "veinlet" and/or free vein. If things were as simple as that, namely the presence of an ordinary semi-dominant mutant (net), argues the author, network should at once be extracted from extra vein flies, which means that all flies showing extra veins should segregate net in the next generation. This is not the case (of course, because extra veins from nature are only rarely net heterozygotes, but usually blistered bs; no real net F_2 was made, see no. 3) and therefore the appearance of network is "connected with a certain

[3] Dubinin makes the erroneous claim that there are no plexus producing mutants outside the second chromosome. Actually a number of them are known, e.g. Beaded, frequently producing a veinlet.

consolidation of hereditary materials." One should read twice this phrase: The extraction of a well known mutant (net), which is 100% penetrant and expressive, and segregates as a single Mendelian trait as found also later on in this work, requires "a certain consolidation"! The failure of extracting net in the first generation due to its relative rarity—see above points 2 and 3—is not a chance result but one of greatest importance! The presence of bs phenotypically identical with net in the heterozygote, which is reported a little later, is passed over in silence. To state the immense error again, the simultaneously present mutant bs has the same or similar phenotype as net if heterozygous or in the presence of modifiers. By breeding from extra vein flies he may have bred from 2 heterozygous bs, or from one heterozygous bs and one heterozygous net, or a combination of both mutants in the same chromosome, easily accomplished by almost free crossing over as net is located at one end and bs at the other end of the chromosome. The following generations may continue so until by chance, in the first or any other generation, two net heterozygotes are mated. It was just bad luck based upon the admitted rarity of net compared with bs that he did not get net in the first generation though he obtained it in the second! More of this below.

12. We actually are told (p. 36) that the original elements for the character network "are units which at first produce only free veinlets. As selection proceeds, this type of variation starts combining with the variant veinlets, a quantitative increase in these traits in the form of lengthening of the extra veinlets appears, a reticular structure follows . . . and finally the complete network is realized." Sometimes this happens this way, *"at other times in rapid jumps,"* which, it seems, does not disturb the author. (See under 11 what really happened. I repeat: a standard mutant net with simple Mendelian behavior as found also by the author in his localization experiments is slowly built up from minor elements, but sometimes in a jump! More of this below, point 14.)

13. For the trait extra veins, it is now stated (p. 38) that in the majority of cases the lines were established in the course of the first five generations of selection (actually about 5/6 of all positive lines). The lines soon became stable and no more selection succeeded. Here stands the remarkable phrase, "This shows, to some extent, direct segregation as well as consolidation based on a simple recombination of the originally heterozygous material." This phrase is also worth reading twice. The facts given state that in 5/6 of these lines a segregant has been isolated (presumably blistered). But in some lines—the remaining 1/6—extra veins appeared only in the 7th, 8th, 9th 11th, 12th, 14th and 22nd generations. This is the basis of the second half of the phrase just quoted. Of course we do not know what happened here as no genetic check was made. But it is known that blistered overlaps normal in heterozygous condition. This as well as the occasional presence of a minus modifier, later removed by segregation or crossing over, would have sufficed to explain the result. Whatever the explanation the fact remains that nothing was done but to extract blistered from the lines.

14. On p. 39 ff. the selection for "network" is analyzed. Here the remarkable phrase is found: "As a rule, selection in these lines is more effective, in many cases leading to a lightning increase in extra venation, transforming the line in rapid steps." Put into plain language this means that "as a rule" the mutant net is easily extracted when present. A number of examples given show that true breeding net was found in the 2nd, 3rd or 4th generation. As the selection was made from flies with veinlets in the first generation and these would be heterozygous net, or homo- and heterozygous blistered, net being the much rarer mutant, this is what is expected for the extraction of net from the population. One case is mentioned in which veinlets were mated only in the 3rd generation and two generations later net was extracted. As heterozygous net overlaps also with normal there is nothing unexpected here.

At the end of this section it is added that in a number of lines complete "consolidation" was not obtained but different degrees of "network" segregated. It is impossible to tell what this means. We do not know whether this happened in mass selections or in one pair selections, both of which were used; no genetic check was made whether here the mutant net was present or a higher allele of blistered which also produces "network" etc. It must be added here that some of the differences of degree in "network" illustrated by the author are known to occur between right and left wings of the same individual. (See my photos 1945.) In spite of the foregoing clear statements by the author that he had "as a rule" simply extracted net and blistered from the population, the paragraph ends with the statement, "All of this material shows that the character of extravenation, ubiquitously spread in natural populations, is based on a complex polygenic system of heredity!"

15. Contrary to the actual facts found by the author himself (see 14) the next chapter is opened with the statement (p. 48) that the

appearance of network "was the result of selection which accomplished a gradual integration of the hereditary bases for extra venation." But a few pages later it is finally and belatedly reported that the type "network" is actually the standard mutant net. This, however, is known to have appeared repeatedly with 100% penetrance and expressivity without selection, and the author's own crossover experiments prove a simple Mendelian behavior of the extracted net. In view of the just quoted conclusion the author now is looking for the polygenic systems in all chromosomes. But the *genetic test which was now finally made showed that only the second chromosome was involved.* "Perhaps we had misinterpreted the results of selection and in reality these variants are not polygenically based. (A sudden flash of insight!) In the contrary case we would find a remarkable picture of the concentration of a complex physiological hereditary system within the limits of one chromosome." The hyperselectionist is of course unable to accept the simple solution which he himself had proved beyond a doubt, and he continues to look for another type of complicated set-up.

16. Crossover experiments with marked second chromosomes were made. They revealed first that the type network is the old mutant net (2; 0.0), further that at least one mutant at the other end of the chromosome was present which was *assumed* to be blistered (plexus, balloon and Plexate are not mentioned as possibly present). In the different crossover chromosomes (made homozygous for the test) the expression of net was weakened, and, in addition, the whole series of variants of extra venation *are said* (see below) to appear. This is interpreted to mean that loci over the whole 2nd chromosome are needed for full expression of network. But only a few lines earlier it is stated (p. 50) that chromosomes homozygous for the markers but with the left end from the stock were pure net! Then on p. 36 it is found that a locus like blistered (balloon, plexus?) at the other end of the chromosome is involved. But it does not occur to the author that in his different crossover classes net or blistered or both could be present, not to speak of other mutants like plexus and balloon which might have been present but were not tested. Of course the markers might have had an additional modifying influence which should have been analyzed individually. One of them was dumpy which I found formerly (1945) to be a minus modifier for extra venation.

But actually the description of the findings is completely nullified by the detailed data. It is worth while to look at the illustrations for this experiment and to compare them with my photos 1945. The result is simply stunning. The claim was made that the whole series of transitions is present. Actually, 3 wings are net, and they are within its range of variation which is even found between two wings of the same individual (see my photos). One wing is net depressed by dumpy (very typical: two of the pictured wings are dumpy which is known to dilute plexation). The rest are blistered types with an without dump. I recommend a good look at these pictures and a comparison with my photos in order to realize the discrepany between claims and facts. Thus the crossover experiments show nothing but the presence of net and blistered (maybe also the nearby px and ba), which may be extracted, plus a little modifying influence of the markers used, especially dumpy. It is incredible that the author, at this point, did not tear up his paper.

The utterly simple situation then is that of a few semidominant mutants floating in a population. To one not acquainted with their phenotypes this is a little complicated by the fact that they are located in the same chromosome, thus allowing for a number of homozygous and heterozygous types, and is further complicated by the fact that some of these phenotypes are identical so that one does not know what is selected before isolating the mutants. But the hyperselectionist author intent on finding a wonderful and complicated system of selected adaptation concludes to the contrary (p. 56) in the face of his own evidence: "These facts leave no doubt that in natural populations the hereditary basis for extra venation, which is so readily affected by selection lies in a complex polygenic hereditary system. . . . The main property of this complex hereditary system is the localization of the genes affecting extra venation within the limits of the second chromosome. This shows that we have encountered a phenomenon of evolutionary intrachromosomal integration of physiologically similar heredity, the complex of which determines the directed variability of traits in natural populations." A very good example of the abysses into which the blindness of wishful thinking leads.

18. It is hardly imaginable that an old *Drosophila* worker like Dubinin did not see what he was doing when he "selected" the net mutant sometimes in the second, sometimes in the third or later generations. Knowing, though belatedly, that the phenotype of heterozygous net overlaps with that of blistered, both present in his line (and in the same chromosome), it was a piece of elementary genetics to realize that he might mate two net heterozygotes or one net heterozygote with a blistered homozygote, etc., and thus was bound to get

the results obtained. But obviously he was so blinded by his enthusiasm for selecting in a polygenic system that he missed the simple fact which his results prove beyond a doubt. Or did he really miss the point completely? Reading carefully pp. 41 ff., I cannot help feeling that at this point, after he had done all the misleading selection work, without genetic check, which was now done finally, he suddenly realized that his beloved polygenes were swimming away, leaving only two well known mutants in one chromosome. Being almost religiously convinced of the correctness of his former analysis he set out now to find his polygenes within the second chromosome. He did it with rather unorthodox crossover experiments, without first isolating the two mutants near the ends of the 2nd chromosome and combining them severally with the markers, and he did it by misinterpreting the phenotypes. Thus he got results based upon mixtures of one or the other or both of the mutants with the markers, and he misinterpreted this as a demonstration of the polygenes which had to be found for psychological reasons. "Reim dich oder ich fress' dich" is a good German characterization of such psychology.

19. A lengthy discussion of these "discoveries" follows in order to show that a case of adaptive polymorphism has been found in which correlated physiological properties are decisive. The most adapted condition is associated with heterozygosity. (The simple and obvious fact is that homozygous net is less viable, as usual, while the heterozygous combinations of net, blistered, etc., present in relatively small numbers in the population, are harmless and keep floating around.) Then follow lengthy discussions of the importance of the supposed genetic system for the populations. This leads to the conclusion, that "the reason for the existence and origin of the hereditary system which secures the optimal expression of physiological adaptation whose external manifestation is variability for extra venation." And we learn further that these data "touch upon three important problems of evolution: 1) the problem of intra-populational heterosis, 2) the genotypic bases of reversible reactivity of populations and in connection with that question, evolutionary tempos, and 3) the problem of organization of heredity within chromosomes, all of which is discussed subsequently. Thus an erroneous interpretation of a few simple and unexciting facts based in part on insufficient knowledge of the material and, more so, upon a basic hyperselectionist attitude which is on the constant outlook for more and better polygenic systems, and more and better selection, has led to blowing out of nothing a huge evolutionary bubble shining in the most brilliant colors, but due to collapse into nothing at the first objective, close look.

20. This criticism is not presented out of pleasure in debunking the work of an otherwise excellent geneticist. It is inspired by a deep concern (felt also by others) for the direction which some recent evolutionary work has taken towards an extreme hyperselectionism which invents more and more modifier systems in order to describe everything in terms of selection. Other possible explanations which may be simple are not even considered. Under the influence of a clannish enthusiasm which calls this type of work modern genetics, implying that everybody else is backward, this hyperselectionist attack has taken hold of many of the younger generation. It has taken hold of those who do not take time out to evaluate critically what is good and well founded and what is gratuitous interpretation. It has taken hold of those who refuse to acquire first a broad knowledge of the field before becoming blind partisans of one idea, not to mention those who refuse even to look at opposing ideas. The foregoing criticism of the work of a prominent leader of that group should show them the pitfalls into which blind enthusiasm may lead.

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THE STRONGYLOCENTROTIDAE (ECHINOIDEA) OF THE NORTHEAST PACIFIC

Emery F. Swan

On the shores of San Juan Island, Washington, within and slightly below the intertidal zone where suitable substrata and other environmental factors exist, there live large populations of *Strongylocentrotus droebachiensis* (O. F. Müller), *S. purpuratus* (Stimpson) and *S. franciscanus* (A. Agassiz). In nearby deeper waters (from some 18 to 108 fathoms) a light

colored urchin which, in so far as the writer has been able to determine, appears to be *S. echinoides* A. Agassiz and H. L. Clark, can be dredged in large quantities. Although the writer had left the area before they were collected, it should be recorded that numerous specimens of *Allocentrotus fragilis* (Jackson) were dredged about 5 miles off Tatoosh Light, Washington, 48° 23.8′ N., 124° 52.0′ W., 176–170 fathoms, by the research ship "Brown Bear" on August 6, 1952. In view of Vasseur's (1951, 1952) recent papers dealing with the separation of species within the genus *Strongylocentrotus* and the variation within the individual species, the following observations would appear pertinent at this time, even though additional evidence is needed before some of them can lead to justified conclusions.

Color and sex in S. franciscanus: Clark (1948) has suggested that "an investigation into the matter of a possible correlation of color and sex [in this species] might prove rewarding." While there is some intergradation, the vast majority of individuals seen in the vicinity of Friday Harbor can readily be considered as belonging to the dark phase or the light phase. Living dark individuals have spines ranging in color from a deep reddish purple to a very dark maroon. The light individuals have primary spines of salmon to rose shades, with secondary spines varying thru various shades of salmon, rose and light violet to nearly white. Within the months of April, May and early June, 1952, 80 specimens of each phase were collected within approximately a mile from the Friday Harbor Laboratories of the University of Washington. These were individually checked for sex by examination of the fresh gonads. Of the dark individuals 51 proved to be males and 29 females; of the light ones 37 were males and 43 females. Thus it is definitely shown that both sexes are represented in both color phases. An examination of the above figures alone tempts one to suggest a possible sex linkage of the color determining factors, but the facts that the individuals of this species move around considerably and are much more numerous at any given tide level at some times than at others leaves open the possibilities that there may be differences in the wanderings between the sexes and/or between the members of the two phases. This suspicion is intensified by the fact that the ratio of females to males decreased markedly among the collected specimens of the light phase as the season progressed.

Maximum size of S. purpuratus: Mortensen (1943) records a test diameter of 68 mm. as being, to his knowledge, the maximum size attained by this species. On the shores of the San Juan Islands, Washington, specimens of greater size are common. The six largest specimens collected during the winter of 1951–52 were saved and sent to the U. S. National Museum. These ranged in test diameter from 89 to 99 mm.

Natural hybridization between species: Although such specimens probably occur only once among several thousand, the writer has seen a few which, from general appearance, would be judged to be hybrids: *S. purpuratus* × *S. droebachiensis*, *S. purpuratus* × *S. franciscanus*, and *S. droebachiensis* × *S. franciscanus*. The most carefully examined of these was collected intertidally on the west side of San Juan Island, Washington, early in December, 1951, by R. L. Fernald and A. H. Whiteley. Its denuded test is 77-½ mm. in diameter and 37 mm. in height. Such dimensions fall well within the limits of both *S. purpuratus* and *S. droebachiensis* as they occur in this locality. The spines were, except for their lavender tips, of a willow green color, similar to that of the spines of small (i.e. young) *S. purpuratus*. Green specimens considered to belong to the latter species have been discussed by Rathbun (1887) and Mortensen (1943); Clark (1912) and Grant and Hertlein (1938) discuss possible intergradation between the species. Examination of cross-sections of the primary ambital spines of the specimen reveal them to have a central core violet in color surrounded by willow green radial wedges (31–41 per spine with an average for the 20 spines of 34.4). In typical *S. purpuratus* there is a central core of a light brown to brownish green surrounded by violet wedges, which in the ambital spines of specimens of comparable size average between 42 and 50 per spine. The core and material between wedges in *S. droebachinesis* are nearly white, and the wedges are of various shades of brown to yellow green and in specimens of comparable size average 24–28 per spine at the ambitus.

This specimen was a female with small ovaries. In early April, 1952, injection with KCl isotonic with sea water failed to induce spawning even though successful with the majority of individuals of both *S. purpuratus* and *S. droebachiensis*. When the specimen was opened (June 4, 1952) a few eggs appeared ripe and ranged in diameter from 112 to 120 μ. The eggs of *S. purpuratus* from the same region range from 79 to 90 μ, and those from *S. droebachiensis* 150–172 μ. Sperm agglutination tests (cf. Lillie, 1921 and Vasseur, 1951, 1952) were attempted with both species, using egg water of this specimen but no agglutination was obtained. It may well be that this is a result of the slight development of the ovaries and/or the technique used, and no conclusions are considered warranted.

In each ambulacral radius there were several arcs of 7 pore pairs with the majority of other arcs except in the vicinity of the apex and peristome having 6. A single arc of 8 pore pairs was found. 8 or 9 is the general maximum number of pore pairs per arc in *S. purpuratus* and 6 in

S. droebachiensis with 7 occurring only very rarely.

The cleaned test is intermediate in color (depth of green) between the two species; as were the globiferous and tridentate pedicellariae in size. The apical system looks very much like that of *S. purpuratus.* An examination of the parts of the lantern reveals long styloid processes on the half pyramids very similar to those of *S. droebachiensis* and markedly different from the short ones characteristic of *S. purpuratus.* The denuded test, the parts of the lantern, the cut bases of the ambital spines, and a few entire primary spines of this specimen have been deposited in the U. S. National Museum. Mr. Austin H. Clark suggests that this specimen approaches *S. echinoides* and *S. polyacanthus* A. Agassiz and H. L. Clark in some respects. The possibility that it may be an individual of a species not usually found in this geographic area or ecological situation is certainly worthy of consideration, but its higher number of wedges per spine than *S. droebachiensis* is evidence against its being *S. echinoides.*

An ill-fated specimen was collected in the fall of 1950 near the laboratory's observation pier. In life it looked intermediate between *S. droebachiensis* and a light colored *S. franciscanus* in both color and length and arrangement of spines. Its denuded test was 55 mm. in diameter and 28 mm. in height. In each ambulacral radius were several arcs of 7 pore pairs, and the arcs were much more nearly erect than is the case in either *S. droebachiensis* or *S. purpuratus*—a characteristic of *S. franciscanus* which however, at this size, generally has some arcs with 8 or 9 pore pairs. Its sex is not known, and its lantern was lost. The average number of plates per ambulacral row is 25.1 and per interambulacral row 16.7. For *S. franciscanus* from the same area of the same diameter, the numbers of plates are 16.7 ± ca. 0.5 per ambulacral row and 13.3 ± ca. 0.5 per interambulacral row. For *S. droebachiensis* of comparable size from the same area the numbers would be 28.4 and 18.8 with a range of variability of about ± 2. The number of wedges in three of the ambital spines were 41, 38 and 36, all well beyond the normal range for *S. droebachiensis* and below that of *S. franciscanus.* This last character is strong evidence against this indivdual's being a stray *S. echinoides* which had wandered up into the intertidal.

In the summer of 1949 a specimen looking like a typical *S. purpuratus* on one side but with spines long enough for *S. franciscanus* on the other was collected at Iceberg Point, Lopez Island. This was turned over to a student who, at that time, was trying to determine which characters reliably separate the species, and was subsequently lost.

Several other specimens have been seen that were, on superficial characters, impossible to assign to species or to guess as to what hybrid combination they might represent. Also, a number of specimens have been observed that appeared to belong to one species but to have in some characters some degree of resemblance to one of the others. Whether these are simply variants within a species or whether they are individuals whose ancestry includes members of more than one species is not known.

Ecological preferences of the species: The three species *S. purpuratus, S. franciscanus,* and *S. droebachiensis* live together in the lower intertidal rocky areas of the more exposed parts of the west side of San Juan Island and the southern tip of Lopez Island. In the quieter and slightly less saline waters of San Juan Channel, *S. purpuratus* becomes rare, and in Puget Sound proper *S. franciscanus* decreases in numbers until finally *S. droebachiensis* alone is left. In just how deep water these species live locally is not known. *S. purpuratus* is rarely dredged, but this may not indicate its absence. Intertidally it habitually lives in crevices and attaches itself with extreme tenacity to the rocks. Where there is a rocky bottom and currents are moderate to swift, *S. franciscanus* occurs down to at least 50 fathoms. Where they can be seen a few feet below the water level, members of this species occur in greatest numbers on the vertical faces of ledges. Under the same conditions *S. droebachiensis* occurs in maximum numbers on horizontal or sloping rock surfaces, and is frequently found on gravel or mud, occasionally in considerable numbers in eel grass patches. Because of its confusion with *S. echinoides,* the records of distribution of *S. droebachiensis* are unsatisfactory, but specimens, unquestionably *S. droebachiensis,* from at least 70 fathoms have come to the writer. No unquestioned specimens of *S. echinoides* have been seen collected from less than 18 fathoms, and from the deepest (108 fathoms) dredging done from these laboratories in the years 1948–52 this species alone among the echinoids was collected. *S. echinoides,* however, was found in considerable numbers at this depth.

The taxonomic status of the sublittoral light-colored sea urchin: In many areas in depths ranging from 18 to 108 fathoms, especially where the bottom is of gravel, shell, or covered with mussels (*Volsella modiola*), there exist large populations of an urchin which differs from the *S. droebachiensis* of intertidal regions in a number of respects: 1) Their spines are of a very light color, often nearly or really white; 2) The living tissue over the test is of a red to white color rather than dark purple; 3) The primary spines are longer and the secondary spines are very numerous, fine, and short, especially aborally; 4) They have fewer wedges per spine (These, for specimens about 50 mm. in diameter, have on the average 20–22 wedges per primary

ambital spine as compared with 24–28 for *S. droebachiensis*); 5) Smaller and lighter colored globiferous pedicellariae (but of the same general shape); 6) Higher average number of pore pairs per arc (averages of ten specimens with arcs counted as suggested by Vasseur, 1951, being 5.99 for these light specimens with a range of averages per specimen of 5.40 to 6.63 in contrast with *S. droebachiensis* with a mean average of 5.63 and a range of averages of 5.20 to 5.98); and 7) Shorter styloid processes on the half pyramids of the lanterns. These characters, in so far as comparable, agree with Mortensen's (1943) description of *S. echinoides* A. Agassiz and H. L. Clark, and in this paper these light colored specimens are meant when that name is used. Unfortunately, before specimens had reached Dr. Mortensen for confirmation of identification, he had died. Grant and Hertlein (1938) and A. H. Clark (personal communication) doubt that *S. echinoides* is separable from *S. droebachiensis* on the species level.

In an attempt to shed some light on the question as to whether *S. echinoides* and *S. droebachiensis* are specifically separable or identical (cf. Mortensen 1943, pp. 217, 218, 222) preliminary qualitative tests of sperm agglutination by egg water have been made with all possible combinations of *S. droebachiensis, S. echinoides, S. franciscanus* and *S. purpuratus*. Only two moderately ripe males and three such females of *S. echinoides* could be found (April 14, 1952). Hence the results while definite for these individuals cannot unreservedly be considered general for the species. With abbreviations used for the trivial names of the species and Vasseurs' (1951, p. 13) symbols for degree of agglutination the results obtained were as follows:

		Egg water of:			
		droeb.	echin.	fran.	purp.
Sperm of:	droeb.	++	− to (±)	− to (+)	−
	echin.	−	++ to +++	−	+++
	fran.	−	− to +	+++	− to +
	purp.	−	+++	−	+++

The symbols in parentheses involved the use of the sperm of one *S. droebachiensis* collected intertidally and appearing superficially typical for the species in this area except that its primary spines were a little longer than usual and of a decidedly reddish hue. These findings agree essentially with Lillie (1921) in so far as *S. purpuratus* and *S. franciscanus* combinations are concerned; and if *S. echinoides* be exchanged for *S. pallidus* with Vasseur's (1951, 1952) data for *S. pallidus* and *S. droebachiensis*. Heteroagglutination tests between the two pairs of species have never before been performed to the writer's knowledge. Except that the sperm of the above described *S. droebachiensis* was weakly agglutinated by egg water of both *S. echinoides* and *S. franciscanus, S. droebachiensis* used in either direction appeared without effect on any of the other species. One batch of the *S. echinoides* egg water questionably agglutinated *S. franciscanus* sperm. In so far as the individuals used were concerned, there was no doubt about the agglutination of *S. purpuratus* sperm by *S. echinoides* egg water nor for the reciprocal reaction. In view of Tyler's (cf. Tyler, 1949) findings of strong agglutination between *Lytechinus pictus* and *S. purpuratus* it is worthy of note that the latter species should be found to show heteroagglutination with still another species.

It should be noted that in all tests a drop of $CaCl_2$ solution isotonic with sea water was added to the sperm solution before the egg water was added. Because eggs from both *S. droebachiensis* and *S. franciscanus,* unless quite ripe, appeared to yield a water weak in agglutinating power even on sperm of the same species, only fully ripe specimens were used.

The sorting of mixed populations of *S. echinoides* and *S. droebachiensis* has not been attempted nor has the frequency of hybrid individuals been determined. That the *S. echinoides* approach *S. pallidus* (G. O. Sars) in some respects as suggested by Vasseur (p. 96, 1952) cannot be denied, but their identity is not proven. Pertinent to this subject is a collection (made available through the courtesies of the Office of Naval Research, the Arctic Research Laboratory, and Dr. Ira L. Wiggins) of some 70 sea urchins dredged in July, 1951, within a radius of five miles of Point Barrow, Alaska, in water varying from 25 to 28 fathoms in depth. These Alaskan specimens had lanterns proportionately much heavier than those of either the *S. droebachiensis* or *S. echinoides* of the Friday Harbor region. They had an average number of wedges per ambital spine for 49 specimens of from 16.8 to 21.2 with a mean average of 18.5. Ten specimens examined for number of pore pairs per arc gave a range of averages of 5.49 to 6.70 with a mean of 6.10. The primary spines are shorter than those of the local *S. droebachiensis* and even more markedly so than those of *S. echinoides.* The secondary spines are not especially fine. In fact, on all counts that can be checked they agree with Vasseur's description (1951) of *S. pallidus* (G. O. Sars).

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COMMENTS ON EVOLUTIONARY LITERATURE

Ernst Mayr

Evolution in pond snails.[1] It is recognized more and more clearly that evolution has so many facets that it has to be studied in all of its forms before we can begin to generalize safely. And in particular there is great need for comparative studies. Evolution is different in the horses, in birds of paradise and in *Drosophila*. It is different again in freshwater snails, as is evident from an excellent revision of the family Lymnaeidae by Hubendick. The outstanding feature in this family is that the numerous species are very similar, often confusingly similar to each other, while within these species there is great variability from locality to locality. As a result over 1000 names have been given to about 40 valid species.

A detailed analysis of samples from nearly 70 populations of *Lymnaea peregra* shows that the variability within a given population is quite low, that neighboring populations may be very different, and that no clear correlation can be established between certain environmental conditions and definite shell types, although certain shell types occur only in hard water. Anatomical characters, such as the form of the spermatheca, show striking uniformity within each population, but may vary conspicuously between populations.

As in other freshwater organisms, the main question is whether the great local variability has a genetic basis or is due to a non-genetic modifiability by water chemistry, temperature and feeding conditions. The conclusion is that both are involved but that the genetic component is by far the stronger. Furthermore, the morphological uniformity of populations appears to be due to genetic uniformity. The lymnaeids are hermaphrodites and self-fertilization occurs commonly. It is possible, even probable, that most local populations are founded by a single (self fertilizing) individual, which would

[1] Hubendick, B. 1951. Recent Lymnaeidae. Their variation, morphology, taxonomy, nomenclature, and distribution. Kungl. Svenska Vetensk. Akad. Handl., (4) **3**: 1–223, 5 plates, 369 figs.

account for uniformity of most of the populations (some highly variable populations are also known, particularly in the older parts of the range of the species). The coefficient of variability for the length/width ratio of the shell fluctuates between 1 and 4 for most populations. On the other hand dispersal within the total species range seems to be sufficiently frequent to prevent the origin of well defined geographic races.

Hybridization has been observed between individuals of different species of *Lymnaea*. The rarity of hybrids indicates well developed sterility barriers. Not enough is known about the chromosomes to determine whether or not they contribute to cytological isolating mechanisms.

No clear phylogenetic lines can be distinguished within the genus. Every known taxonomic character of shell and internal anatomy varies independently of all the others. No species groups or subgenera can therefore be distinguished.

Race and species formation in free living nematodes.[2] It has been known for some time that the free living nematodes show various puzzling evolutionary phenomena. Lack of knowledge of their systematics has held back an understanding. In recent years H. J. Stammer and his Erlangen group have greatly added to our knowledge and have laid a foundation for more intensive genetic and cytological work. H. Hirschmann describes an interesting case of morphological dimorphism in *Diplogaster lheritieri* and the instantaneous origin of a hermaphroditic species. She finds that most individuals of *D. lheritieri* retain the narrow larval shape of the mouth as adults (*stenostoma*), but some 2–7 per cent metamorphose into a broad mouthed form (*eurystoma*). Some populations produce only *stenostoma*. A change of the culture medium does not result in a shift of the ratio between the two types. Crosses between *Eu* and *St* may result in pure *St* offspring, and *Eu* may occur in crosses *St* × *St*. This would indicate that *Eu* is recessive. However, in crosses *Eu* × *Eu* never more than 50 per cent *Eu* were found and sometimes only *St* (all crosses were done under identical conditions of temperature and nutrition). Inbreeding often results in complete sterility. In many inbred broods disturbances of sex determination occurred (intersexes, individuals with degenerated gonads). In four of the crosses small, slender hermaphrodites appeared which were proterandric and self fertilizing. These stocks produced also small, residual males. Numerous attempts of crosses between the hermaphrodites and parental strains were without results. In view of this complete reproductive isolation, the hermaphrodites are described as new species (*"biformis"*). On several occasions such hermaphrodites were also found in the wild. Each of four more closely investigated wild strains of *biformis* differed in the percentage of *eurystoma* in adults and larvae and in the frequency of residual males. Each strain probably originated independently of the others. All this is very puzzling and cannot be discussed profitably until the cytology of these forms has been analyzed. Quite a few similar cases have been described in the nematodological literature, where of a pair of exceedingly similar species one is sexual and the other an obviously derived hermaphrodite.

Stammer[3] points out that in these nematodes there is an uninterrupted series from normal, sexually reproducing species through hermaphrodites with and without functional males to parthenogenetic species. In some genera and groups of species only sexual reproduction is known. In others various secondary deviations from normal sexuality occur. Clusters of sibling species occur not infrequently in these latter groups.

Osche[4] gives a thorough and stimulating analysis of systematics and speciation in the difficult genus *Rhabditis*. The process of speciation in the sexually reproducing species is still obscure. No geographic races are, so far, known and several species are known to be virtually cosmopolitan. Sterility barriers develop apparently rapidly and various very similar species are not crossable. In a few exceptional cases there is no sexual isolation, but the sperm fails to fertilize the eggs or the eggs die in early cleavage (*Rhabditis paraciliata* × *R. pseudoxycerca*). This is precisely the condition so often found in *Drosophila*. Races have been described for various species of *Rhabditis*, but subsequent analysis has always shown that either sibling species or intrapopulation variants are involved. The case of the *inermoides* type of *R. inermes* is still obscure. In this species two types of larvae occur, "fiddlers" and "non-fiddlers." [The waving movements of the fiddler larvae above the substrate help them to become attached to their vectors, various kinds of insects.] The two types are completely fertile with each other, and fiddling is dominant over its absence. Yet, there is considerable ecological difference between these polymorph variants. Fiddlers occur primarily on carrion, where they depend on phoresy by carrion beetles, while non-

[2] Hirschmann, H. 1951. Ueber das Vorkommen Zweier Mund-hoehlentypen bei *Diplogaster lheritieri* Maupas, etc. Zool. Jahrb. (Syst.), **80**: 132–170.

[3] Stammer, H. J. 1951. Fortpflanzung und Artbildung bei freilebenden und insektenparasitischen Nematoden. Verh. Deutsch. Zool. Ges. Wilhelmshaven, pp. 433–438.

[4] Osche, G. 1952. Systematik und Phylogenie der Gattung *Rhabditis* (Nematoda). Zool. Jahrb. (Syst.), **81**: 190–280.

fiddlers are found in stables and along muddy shores where passive dispersal is possible without phoresy. In the absence of sexual isolation it is doubtful whether this partial ecological isolation is a case of incipient speciation. The few situations, here quoted, indicate clearly the great potentialities of nematodes for evolutionary research. Nearly all the species can be raised easily on laboratory media and the length of a generation (5 days) is conveniently short. They appear to be ideal laboratory material for genetic research, as previously noted by Dougherty and others.

Evolution in the Indian fish fauna.[5, 6, 7] It is generally acknowledged that the Indian Peninsula was separated from the mainland of Asia during most of the Mesozoic and became attached to it during the Cenozoic. Peninsular endemics may have evolved since the joining or they may antedate it. Hora, in a series of papers, has tried to elucidate the evolution of the peninsular fish fauna, with particular emphasis on the fishes specially adapted for life in torrential streams. Further light on these problems is shed in three recent papers by Hora and his associates. Menon shows that the eastern Ghats, ranges of rather low hills in the eastern Peninsula, could not have served as a pathway of immigration for freshwater fishes. They have no real endemics and have received their more interesting elements during the late Pleistocene as a result of a change of drainage due to a west-east tilting of the Peninsula. Silas analyzes the level of evolutionary divergence of fishes with Malayan affinities. Among the 47 isolates, there are 4 endemic genera and 4 subgenera with a total of 9 species. Eleven other species and 12 geographic races are also endemic. Fifteen isolates do not differ even subspecifically from populations occurring in Assam or farther east. At least two causes are responsible for the different degrees of divergence. There have been different periods of colonization and the earliest colonists had the greatest opportunity to diverge. Unfortunately, Silas fails to specify how long ago in the Tertiary Peninsular India became accessible to freshwater immigrants from the east, as well as to discuss the nature of the antecedent marine barriers. A second cause for uneven rates of evolution is ecological. Fishes of torrential streams are more completely isolated and subject to greater specific selection pressure than fishes of other waters. As a consequence they have a tendency to evolve faster. Hora likewise points out the importance of ecological factors for the immigration itself. During the glacial periods of lowered sea level and increased precipitation, many streams must have shown torrential conditions in areas that are now lowlands. The spread of torrential fishes can thus be explained without recourse to otherwise unsupported mountain building.

Evolution of the Madagascar fauna.[8] The peculiar fauna of Madagascar and the other islands in the western Indian Ocean has often been cited in support of a former Gondwana Continent connecting Africa and India. Millot shows in a thorough study that the actual facts support the Matthew-Simpson contention that these islands never had continental connection. Not only is the fauna of Madagascar itself highly unbalanced and a typical transoceanic invasion fauna, but the fauna of the other islands, like Seychelles and Mascarenes, has no obvious relationship to that of Madagascar. The fallacy of many of the so-called proofs for Gondwanaland is demonstrated.

Allopatric hybridization.[9] The grackle of North America was divided during the Pleistocene into an eastern and a western population. The eastern, the Purple Grackle (*Quiscalus q. quiscula*), centers around Florida and the Carolinas; the western, the Bronze Grackle (*Q. q. versicolor*), is found west of a line extending from Louisiana to Massachusetts. Where the two populations met after the Pleistocene a variable hybrid belt developed which extends from the mouth of the Mississippi along the Appalachians to New England. There is a regular increase of size with latitude, but most populations of the hybrid belt are of larger size (wing length) than to be expected according to latitude. Such a heterosis in natural populations is of considerable interest. The bill length decreases with latitude. The author questions that heat loss through the bill could be sufficiently severe to be of selective significance and ascribes the difference to an increasing proportion of soft food in the more southern popu-

[5] Hora, S. L. 1951. Some observations on the palaeogeography of the Garo-Rajmahal Gap as evidenced by the distribution of Malayan fauna and flora to Peninsular India. Proc. Nat. Inst. Sci. India, **17** (6) : 437–444.

[6] Menon, A. G. K. 1951. Further studies regarding Hora's Satpura hypothesis. 1. The role of the eastern Ghats in the distribution of the Malayan fauna and flora to Peninsular India. Proc. Nat. Inst. Sci. India, **17** (6) : 475–497.

[7] Silas, E. G. 1952. Further studies regarding Hora's Satpura hypothesis. 2. Taxonomic assessment and levels of evolutionary divergences of fishes with the so-called Malayan affinities in Peninsular India. Proc. Nat. Inst. Sci. India, **18** (5) : 423–448.

[8] Millot, J. 1952. La faune malgache et le mythe gondwanien. Mém. Inst. Sci. Madagascar, **A 7** : 1–36.

[9] Huntington, C. E. 1952. Hybridization in the Purple Grackle, *Quiscalus quiscula*. Syst. Zool., **1** : 149–170.

lations. However, a similar change of bill length occurs also in grebes and other birds in which a latitudinal change of food is unlikely. The width of the hybrid belt is narrow in Louisiana and in the southern Appalachians (200 miles) and wide in Alabama and in the northern Appalachians. Other things being equal, ecological factors determine the width of the belt, but genetic factors are probably responsible for the over-all narrowness of such hybrid belts.

Gene flow and adaptation.[10] There has been growing terminological confusion in recent years with respect to gene flow between natural populations. E. Anderson has introduced the useful term introgression for the incorporation of genes of one species into the genome of another species. Lately he and his collaborators seem to want to extend this term to any kind of gene flow between populations, thereby robbing the term introgression of whatever usefulness it had at the beginning. Woodson describes a case of "introgression between the sympatric subspecies *interior* and *terminalis*" of the butterfly weed *Asclepias tuberosa*. From his account it is difficult to understand what he is driving at. The contradictory statement "sympatric subspecies" is not documented, rather there seems to be a cline from luxurious plants with broad leaves and many flowers in the center of the range toward poor plants with narrow leaves and few flowers toward the periphery of the range (Texas and Minnesota), each population, of course, having a normal amount of variability. Only a single interbreeding population seems to occur at any one place. The picture thus presented by *Asclepias* is that described by Rensch[11] (1932) more than twenty years ago for land snails which reach maximum size in the optimal part of the range and decline in size toward the periphery. This is generally acknowledged to be due to the interaction of genetic factors (mutation, recombination) and ecological factors (selection, gene flow). Woodson's analysis of the natural populations and their clinal changes is admirable, but I can see no particular advantage in the attempt to introduce new terms for phenomena for which a well established terminology is already available.

Secondary interbreeding.[12] The Yellow-bellied Sapsucker (*Sphyrapicus varius*) has four subspecies, forming two groups. The western group, *daggetti* (Sierra Nevada) and *ruber* (Oregon to Alaska), has more red in the plumage and little or no sexual dimorphism. The more easterly group, *nuchalis* (primarily Rocky Mountains and eastern slope of Cascades) and *varius* (from the Yukon across the north of the continent to New England and the Appalachians), has pronounced sexual dimorphism. The two groups are often considered species. However, there is much interbreeding where the various forms meet. It is complete between *ruber* and *daggetti*, frequent between *daggetti* and *nuchalis* and between *varius* and *nuchalis*, and rare or absent between *ruber* and *nuchalis* and between *ruber* and *varius*. Although there are no significant differences in the life histories, nor any evidence for reduced fertility or viability of intermediates, there is apparently sufficient preferential mate selection and ecological difference in the contact zone between the eastern and western group to prevent complete amalgamation. Presumably the four subspecies formed, on the basis of pre-existing differences, during the height of the Wisconsin glaciation. The great similarity between *nuchalis* and *varius* suggests to me that *varius* may have spread only rather recently into the eastern portion of its range. The most interesting aspect of this thorough study is that in spite of extensive, if not absolute, fertility there is no breakdown of the various gene complexes in their long zone of contact. The change from pure *ruber* territory to pure *nuchalis* territory occurs in a band only a few miles wide.

A narrow hybrid belt.[13] The eastern house mouse (*Mus. m. musculus*), which is light-bellied, and the western house mouse (*M. m. domesticus*), which is dark-bellied, meet in Jutland (Denmark). The hybrid zone is, as in so many zones of allopatric hybridization, remarkably narrow. Within a few miles one may pass from an almost pure *musculus* to a prevailingly *domesticus* population. The hybrid zone coincides with a zone of a certain amount of climatic change. Relative length of tail is another character changing within this belt. More detailed analysis shows that every population has certain characteristics of its own.

Size trends in recent human evolution. Hooijer in a series of papers has shown that in Indonesian mammals, as was previously demonstrated by Degerboel for Denmark, there has been a diminution of size since the Pleistocene. His application of this thesis to man[14] has not

[10] Woodson, R. E., Jr. 1953. Biometric evidence of natural selection in *Asclepias tuberosa*. Proc. Nat. Acad. Sci., **39**: 74–79.

[11] Rensch, B. 1932. Oekologische Molluskenstudien I. Zeitschr. Morph. Oekol., **25**: 757–807.

[12] Howell, T. R. 1952. Natural history and differentiation in the Yellow-bellied Sapsucker. Condor, **54**: 237–282.

[13] Ursin, E. 1952. Occurrence of voles, mice and rats (Muridae) in Denmark. Vidensk. Medd. Dansk. naturh. Foren., **114**: 217–244.

[14] Hooijer, D. A. 1950. Fossil evidence of Austromelanesian migrations in Malaysia. Southwest J. Anthr., **6**: 416–422.

remained unchallenged.[15] To be sure, the teeth of mid and late Pleistocene human fossils on Java average larger than those of the present population, but there remains the question whether this is due to an evolutionary reduction in size of a stationary population, or due to replacement of a large sized population by smaller sized later immigrants, as assumed by the majority of anthropologists.

As good as Hooijer's evidence is for the mammals,[16] it seems dangerous to extend it to man. In living mammals there is usually a regular cline of decreasing size from the continent of Asia through Sumatra to Java. In human races there is usually great irregularity, with considerable size differences often even between neighboring tribes. The very extensive migrations and colonizations in man, well known to Hooijer, are another phenomenon by which man differs drastically from all other mammals (except maybe the domestic rats). Furthermore, there is much indirect evidence available that (genetic) size in man depends much less on climate than on general living conditions (a condition apparently paralleled in certain rodents and insectivores). Consequently, it seems to me that von Koenigswald is justified in questioning the propriety of applying the mammalian findings to man.

Selection in blood groups? There is much in the distribution of the frequencies of blood group genes which suggests differences in the selective value of various genotypes. To find proof for this assumption has turned out to be difficult. To begin with, most investigators have looked for viability differences between the genes themselves, while on the basis of the theory of balanced polymorphism one would expect a superiority of heteroxygous genotypes. Which investigator has compared age classes of the same population for differences in the frequency of AB versus AA or BB? The second difficulty is that many of the sera are not entirely specific and that it is difficult if not impossible to distinguish between the genotypes AA and AO, or BB and BO. The third difficulty is that the selective value of a given genotype is affected not only by the external environment but by the genetic background as well. If, for instance, Tibetans should have a similar frequency of B as south Indians, this would not in the least prove that B is a neutral gene. It might rather indicate that the interaction of the physical environment of the Tibetan highlands with the residual genetic background of the Tibetans, and the interaction of the physical environment of tropical southern India with the residual genetic background of the southern Indians would result in similar B frequencies. For all these reasons similar gene frequencies can not be accepted uncritically as proof for close relationship. As valuable as the data are which indicate relationship between peoples, on the basis of their blood group genes, it is equally important to make specific investigation of possible viability differences of various genotypes and to look for any deviation from the expected frequencies. Lahovary[17] points out the interesting fact that blood group O has a tendency to increase in frequency in proportion to the isolation and numerical weakness of the community concerned. In Switzerland, for instance, the frequency of O is around 45 per cent in the plains and at the mouths of valleys, but it rises with elevation, reaching 50 per cent, then 55 per cent, and finally 60 per cent in the highest and most secluded villages at the upper ends of the valleys. This is equally true for German Swiss communities on the north slope of the Alps and for Italian Swiss communities at the south slope. These populations are ethnically not different from the lowland population, in fact many of them were colonized in historical times by settlers from the lowlands. Obviously then, the difference is a matter of selection. Perhaps, as I have suggested elsewhere, O is one of those genes which is superior in homozygous conditions, while A and B are superior in heterozygous combinations. A similar increased frequency of O is found on islands, in deserts, and presumably among the small bands of North Asiatics that colonized North America. The differences in the viability of the blood group genotypes can no longer be ignored.

Population hybrids.[18] In the work of Dobzhansky and his associates on gene arrangements the technique up to now consisted in the comparison of the viability of homozygotes with that of heterozygotes. Vetukhiv in an ingenious experiment tests the relative viability of homozygotes from various localities and of F_1 and F_2 hybrids between them. The viability is measured in terms of percentual survival in competition with a standard mutant stock. The results show not only that each local population has its standard viability, but that the viability of the F_1 is normally considerably greater and that of the F_2 considerably less than that of the parental stocks. The reduced viability of the F_2 is consistent with the hypothesis that recom-

[15] von Koenigswald, G. H. R. 1952. Evidence of a prehistoric Australomelanesoid population in Malaya and Indonesia. *Op. cit.*, **8**: 92–96.

[16] Hooijer, D. A. 1952. Austromelanesian migrations once more. Southwest J. Anthr., **8**: 472–477.

[17] Lahovary, N. 1953. Bioserological methods of human classification. Science, **117**: 259–269.

[18] Vetukhiv, M. 1953. Viability of hybrids between local populations of *Drosophila pseudoobscura*. Proc. Nat. Acad. Sci., **39**: 30–34.

bination has broken up the co-adapted gene complexes. The greatly increased viability of the F_1 is puzzling but seems to support the concept that heterozygosis is *per se* a viability stimulus.

Inversions and crossing over.[19] Inversions succeed in keeping co-adapted gene complexes together through drastically reducing crossing over. This advantage is, however, coupled with the disadvantage of a reduction in the frequency of recombination. Since evolutionary plasticity, in turn, depends on genetic diversity produced by recombination, the acquisition of inversions appears at first sight to be a mixed blessing. Carson shows, however, confirming and extending the findings of earlier *Drosophila* workers, that the presence of inversions in one chromosome may lead to a drastic increase in crossing over in other chromosomes. In *D. robusta*, for instance, double structural heterozygosity in chromosome X is correlated with a significant increase of crossing over in the section between the two second chromosome inversions. Structural heterozygosity in the right arm of chromosome 3 intensifies these interchromosomal effects. They are also affected by genic constellation. Carson suggests that chromosomes may be functionally subdivided into sections in which co-adapted gene combinations are preserved (within inversions) and other sections in which crossing over can take place freely. In this way the species may exploit the advantages of adaptive chromosomal polymorphism and at the same time retain evolutionary plasticity through a high degree of recombination in structurally homozygous chromosome sections.

Evolutionary inventions.[20] Evolutionary progress is often measured in strictly morphological terms, such as body size, brain size, modified extremities or integument. It required the development of a great body of biochemical facts before it was realized that much of this morphological progress is made possible by ingenious biochemical "inventions." Granick, who himself has made major contributions to this field, unfolds the fascinating story of the successive series of inventions in the iron metabolism of vertebrates. As the metazoa increased in size not enough oxygen reached the cells by diffusion and blood enzymes and blood circulation had to be invented to bring oxygen to the cells. Biologically huge masses of iron are required to make adequate amounts of hemoglobin. The acquisition of ferrous iron from the ferric complexes in which it is found in the food is an acute problem. Granick proposes that the hydrochloric acid in the stomach provides for the necessary chemical environment to permit this iron intake. Indeed this may have been the primary function of hydrochloric acid before it was utilized in other digestive processes. Another invention has to do with absorption of ferrous iron by the mucosal cells of the intestines, still another with the transport of iron to its storage place (liver) and its place of utilization (bone marrow) by a special protein (siderophilin), finally another with the storage of reserve iron in the form of ferritin.

The point of this fascinating story is that any major biochemical invention, let us say like hemoglobin, requires for full usefulness a whole set of additional inventions, which will affect, directly or indirectly, the physiology and biochemistry of the organism. This, in turn, will favor a genetic instability until all these inventions have been successfully incorporated into the gene complex. As a byproduct of this genetic reorganization there will no doubt appear many morphological changes, some directly adaptational, some phenotypically neutral. It would not seem to be too far fetched to believe that the origin of some novel "types" of animals observed by the students of phylogenies may have been initiated by a biochemical invention. The early period of adjustment to the new internal situation might manifest itself to the comparative anatomist as a period of eruptive evolutionary change. Since the morphological phenotype is merely a byproduct of genes and their physiology, it is obviously impossible to expect a complete understanding of evolutionary changes merely from an analysis of morphological changes. The spread of evolutionary thinking among the biochemists is likely to shed unexpected light on many situations so far puzzling to the evolutionist.

The origin of life.[21] How life originated in the primeval organic "soup" is a problem which it may never be possible to solve completely. Indeed, half the problem is, how to define "life" at this stage. Lanham presents a revised form of Oparin's hypothesis that a system of self-reproducing colloidal droplets preceded the origin of self-duplicating nucleoprotein molecules. He outlines a series of definite steps by which this evolution might have proceeded. A discussion of this problem seems particularly timely in view of the recent studies on lysogenic viruses.

Randomness of mutations.[22] What constitutes randomness of a gene mutation has been interpreted in many different ways. It is now

[19] Carson, H. L. 1953. The effects of inversions on crossing over in *Drosophila robusta*. Genetics, **38**: 168–186.

[20] Granick, S. 1953. Inventions in iron metabolism. Amer. Nat., **87**: 65–75.

[21] Lanham, U. N. 1952. Oparin's hypothesis and the evolution of nucleoproteins. Amer. Nat., **86**: 213–218.

[22] Demerec, M., *et al.* 1952. Bacterial genetics. Carnegie Inst. Washington Year Book, No. 51, pp. 193–198.

admitted that the mutation frequency is different at different loci and that the range of possible mutations at a given locus is severely limited. Furthermore, X-ray and U. V. may induce different mutations or the same mutations in different frequencies. Demerec and his collaborators have now produced evidence which narrows down the randomness of the mutation process even more. Each gene was shown to react to the mutagen in a specific manner. Five among 35 strains of *Escherichia coli* were found to be mutagen stable. In strain 12–72 which was both leucine and phenylalanine dependent, treatment with manganous chloride induced more leucine reversions (594 : 11, while ultraviolet treatment produced more phenylalanine reversions (100 : 57). Thus the action of mutagens on genes may be quite specific. Various experiments indicate that the mutagens do not act on the locus directly, but rather through the metabolism of the cytoplasm or of the nucleus. So far, all known mutagens are rather "violent" agents, yet the former concept that mutation was a strictly autogenous process has become increasingly untenable since Muller's demonstration of X-ray induced mutations. But how far the possible effect of cell metabolism on mutation rate extends is something about which we know as yet exceedingly little.

Polyploidy in lepidoptera?[23] The haploid chromosome number of most lepidoptera studied cytologically is 29–31, but a few species deviate so greatly that some authors have assumed polyploidy. Suomalainen produces good evidence for a different interpretation. It seems highly probable that lepidoptera have a diffuse, non-localized kinetochore, and that fragmentation or fusion of chromosomes does not interfere with mitosis. The larger size of the chromosomes in the species with few chromosomes, and correspondingly the small size of chromosomes in species with many chromosomes, indeed supports the idea that differences in chromosome number are due to fusion or fragmentation, rather than evidence for polyploidy.

Polyploidy in a mammal.[24] Since the occurrence of polyploidy among sexually reproducing animals is still vigorously denied by some of our leading cytologists, there is much interest in every newly reported occurrence. Sachs can confirm the findings of Matthey that the hamsters *Cricetus* and *Cricetulus* have a diploid chromosome number of $2n = 22$, and those of Husted and Matthey that the Golden Hamster (*Mesocricetus auratus*) has a chromosome number of 44. Polyploidy is also indicated in the gerbil genus *Tatera* where a Ceylon species has $2n = 72$, and a South African species $2n = 34$. Various reasons have been cited in the past why polyploidy should be impossible among animals. The author attempts to demonstrate the invalidity of these arguments. Nevertheless, chromosome number itself is not conclusive, unless supported by additional evidence, such as doubling of the amount of nucleic acid and demonstration that intermediate chromosome numbers in related species are absent.

Evolution in a West African archipelago.[25] The bird fauna of four volcanic islands in the Gulf of Guinea illustrates many evolutionary phenomena also known from other archipelagos. Depending on the distance from the mainland 33–57 per cent of the species are represented by endemic forms; one island is continental (one endemic species), the other three islands are oceanic (17–30 per cent of species endemic). Every level of evolutionary differentiation exists, from complete identity with mainland populations to 4 endemic genera. No conspicuous trends in color variation are discernible, except that the colors are often duller and the plumage soft and lax. As far as size is concerned, there is a tendency toward an increase, particularly among the outer islands. An even greater percentage of forms has a relatively larger bill. No single explanation for these tendencies seems possible. There are several cases of double colonization, in which the first arrival is always the larger species.

Evolution of parental care.[26] An increasing amount of attention has been paid in recent years to the evolution of habits and behavior patterns. In this monograph Kendeigh studies quantitatively the share of the sexes in incubation and feeding of the young in birds, the rhythmic alternation between periods spent on and off the nest, and the effect of physiological and environmental conditions on these activities. There is a basic pattern for every species, and often a similar pattern for a whole genus or a family, but also there is a great deal of individual variation. In fact, the variability in some species is quite astonishing. Although not all of this has a genetic basis, some of it undoubtedly has, and it is evident that it forms excellent material for selection to work on. To explain the evolution of behavior, it is most important to study the amount of variability of the behavior patterns in a single population, such as Milani has done for *Drosophila*.

[23] Suomalainen, E. 1953. The kinetochore and the bivalent structure in the lepidoptera. Hereditas, **39**: 88–96.

[24] Sachs, L. 1952. Polyploid evolution and mammalian chromosomes. Heredity, **6**: 357–364.

[25] Amadon, D. 1953. Avian systematics and evolution in the Gulf of Guinea. Bull. Amer. Mus. Nat. Hist., **100**: 393–452.

[26] Kendeigh, S. C. 1952. Parental care and its evolution in birds. Illinois Biol. Monogr., **22**: 1–356.

As with all evolutionary trends in birds, there has been much parallelism with respect to the evolution of special patterns of parental behavior. In Proavis, as in her reptilian relatives, the female presumably took care of the brood. In the living "primitive" birds both sexes share in incubation and care of the young. Kendeigh makes a fairly good case for the thesis that uniparental care is, among living birds, a derived condition. In the majority of the cases it is the female which takes over the major share, but there are quite a few groups of birds where it is the male. In all these cases this seems to have happened independently.

Physiological characters in evolution.[27] The differences in water requirements between various species of rodents are highly significant. Voluntary water consumption ranged from no water at all by some pocket mice (*Perognathus penicillatus eremicus*) to 10.85 cc. daily by some voles (*Microtus pennsylvanicus*), with *Peromyscus* intermediate. Tolerance to the absence of water runs parallel. As might be expected, the tolerance of each species and subspecies is correlated with the aridity of its habitat. Selection for the ability to survive a deficient water supply must have played an important role in the evolution of the water metabolism of these rodents.

Sex determination and reproduction.[28] In terrestrial isopods many puzzling sex ratios have been found together with parthenogenesis. De Lattin's analysis of extensive breeding experiments of *Cylisticus convexus* indicates that sex chromosomes are not involved in sex determination, rather that sex is determined by two separate loci, one of which intensifies the action of the other. He believes that this interpretation is also consistent with the data of Howard and Vandel on other species.

Population fluctuations in a mammal.[29] In a large population of dormice (*Glis glis*) most (750) individuals were marked over a period of five years. Litter size fluctuates between 2 and 9, and mean litter size from 2.0 to 6.4 per year. Dormice are very sedentary, and the same individual may be found in the same nest box for years, although shifts up to 1200 meters have been recorded. Population size fluctuates dramatically from year to year. The number of adults in the study area was: 142 (1949), 23 (1950), 44 (1951), 47 (1952); in the same years that of the young: 242, 11, 179, 90. There were no visible differences in the environment in the years of observation and the causes for the changes are not yet understood.

A species population.[30] It is only rarely possible to determine the total population of a species. In birds such data are available only for a few colonial species, like the gannet, or to vanishing species, like the whooping crane. We now have an accurate count for Kirtland's Warbler (*Dendroica kirtlandi*). The breeding range is an area 64 × 78 miles in the jack pine country of the lower peninsula of Michigan. Teams of observers checking every suitable area found 432 singing males, which corresponds to a total species population of less than 1000. Nearly all birds were found in nearly homogeneous stands of *Pinus banksiana* between the heights of 5 feet and 20 feet.

Three dimensional allopatry.[31] If plotted on a map various species and subspecies of the bathypelagic fish *Chauliodus* appear to have broadly overlapping ranges. However, if the individual catches are analyzed with respect to the water mass in which they were taken, it is found that each population is restricted to certain water masses identified by their temperature-salinity characteristics. The major concentrations of most populations are allopatric, except for a considerable overlap between *Ch. sl. secundus* and *Ch. sl. sloanei* in the Indian and Pacific Oceans.

Origin of cave animals.[32] It has often been doubted that mutation pressure alone was sufficient to explain the loss of pigment and eyes in cave animals. The authors present a series of experiments and observations which show that in *Asellus aquaticus* there is very definite habitat selection according to pigmentation. Among heavily pigmented individuals a higher percentage is photophil, pale individuals include a higher percentage of photophobs. This differential preference has selective significance since pale individuals have higher mortality when exposed to light than dark individuals. Similar observations were made on other species. The origin of cave characters would certainly be greatly facilitated if the initial populations had already a favorable genetic background for the acquisition of further cave characters, as well as a definite preference for the cave habitat.

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[27] Lindeborg, R. G. 1952. Water requirements of certain rodents from xeric and mesic habitats. Contr. Lab. Vert. Biol., Univ. Michigan, **58**: 1–32.

[28] de Lattin, G. 1952. Zur Vererbung der Monogenie von *Cylisticus convexus* Deg. Z. ind. Abst. Vererb., **84**: 536–567.

[29] von Vietinghoff-Riesch, A. 1952. Beiträge zue Biologie des Siebenschläfers (*Glis glis* L.). Bonner Zool. Beitr., **3**: 167–186.

[30] Mayfield, H. 1953. A census of the Kirtland Warbler. Auk, **70**: 17–20.

[31] Haffner, R. E. 1952. Zoogeography of a bathypelagic fish. Syst. Zool., **1**: 112–133.

[32] Janzer, W., and W. Ludwig. 1952. Versuche zur evolutorischen Entstehung der Hochlentiermerkmale. Z. Vererbungslehre, **84**: 462–479.

A mollusc parasitizing a jellyfish.[33] Exactly 100 years ago H. Müller discovered that a small medusa is normally attached to the foot gland of the nudibranch *Phyllirrhoe bucephala.* This medusa was described by Krohn as *Mnestra parasites* and considered a parasite on the snail. Ankel is now able to show that the reverse is the case (after some earlier authors had suspected it). The veliger larva of *Phyllirrhoe* lives as commensal inside the medusa of *Mnestra* and lives on the food caught by the medusa. When it gets too large the young snail crawls to the outside and attaches itself with the foot gland to the manubrium of the medusa which is thereby forced to starve. The nudibranch probably lives on siphonophores from this point on, but many details of the life cycle are still obscure. How such an adaptation could have evolved seems difficult to understand. However, a related genus *Cephalopyge* attaches itself with its foot gland on colonies of the siphonophore *Halistemma.*

Sibling species in Japanese freshwater fishes.[34] Two "races" of the Striated Spinous Loach (*Cobitis taenia striata*) are described which occur sympatrically in three rivers in southwestern Honshu. The author reaches the evidently correct conclusion that these "races" are full species, since the hybrids are sterile, there are no intermediates in nature, there are physiological differences (e.g., oxygen consumption of embryos) and differences in habitat preferences.

Sexual isolation.[35] Attempts to induce copulation between males and females of closely related and similar species of *Agelenopsis* were quite unsuccessful, even though their courtships are very similar.

Man's ancestors.[36] Robinson presents good evidence that the multitude of genera of Australopithecines proposed by Broom includes indeed different types. He considers them contemporary sympatric species. What is more, some of the fossils found outside of South Africa cannot be clearly distinguished by morphological criteria from some of these Australopithecines. For instance, the East African fossil called *Meganthropus africanus,* consisting of a fragment of the maxilla with P_3 and P_4 falls within the range of variability of *Plesianthropus,* and the Javan *Meganthropus* falls within that of *Paranthropus crassidens.* This indicates that forms with Australopithecine characters existed not only in South Africa, but also in East Africa and Java. This new evaluation of *Meganthropus* is particularly interesting in view of the repeated insistence by Weidenreich and von Koenigswald that it was very close to *Pithecanthropus.* It is evident that Robinson considers the South African ape-man a rich group, one branch of which gave rise to modern man, while other branches continued to exist and to coexist with modern man. The serious problem of competition which this proposal raises is not discussed.

PLANT PHYLOGENY AND EVOLUTION

G. L. Stebbins, Jr.

One of the major objectives of evolutionists is to learn how to interpret the past in terms of evolutionary processes now going on. In the animal kingdom, we have progressed far in this direction, as is testified by numerous articles which have appeared in the present journal, and particularly by the publications of Dr. G. G. Simpson. Evolutionists studying the plant kingdom, however, have been by no means so successful. This fact becomes evident after one studies the contents of a volume containing the proceedings of an International Colloquium on Plant Evolution and Phylogeny held in Paris in May, 1952.[1] Of the eighteen major contributions to this colloquium, thirteen pay little if any attention to the basic evolutionary processes recognized by modern experimentalists, namely mutation, gene recombination, natural selection, and reproductive isolation. These articles deal exclusively with descriptive morphological, paleontological, or ontogenetic data or with idealistic abstractions and speculations based upon such data. So far as their ideas and thinking are concerned, they could have been written just as

[33] Ankel, W. E. 1952. *Phyllirrhoe bucephala* Per. and Les. und die Meduse *Mnestra parasites* Krohn. Pubbl. Staz. Zool. Napoli, **23**: 91–140.

[34] Minamori, S. 1952. Physiological isolation in Cobitidae. I. Two races of the Striated Spinous Loach with special reference to the difference in their embryonic respiration. Jour. Sci. Hiroshima Univ., B, **13**: 199–212.

[35] Gering, R. L. 1953. Structure and function of the genitalia in some American agelenid spiders. Smithson. Misc. Coll., **121** (4): 1–84.

[36] Robinson, J. T. 1953. *Meganthropus,* Australopithecines and hominids. Amer. Jour. Phys. Anthr., **11**: 1–38.

[1] Évolution et Phylogénie chez les Végétaux Colloques Internationaux du Centre National de la Recherche Scientifique, Vol. 41. 320 pp. Paris, 1952.

well in the nineteenth century as in the twentieth. One contribution, that of the Abbé Jean Augier, is a frank avowal of Lamarckism, though with no new data to supplement those which have already been examined, found faulty, and discarded by other evolutionists.

Of the remaining four contributions two, those by W. M. Hiesey and C. D. Darlington, contain summaries of recent information about race and species differentiation, with Darlington paying particular attention to alterations in chromosome number. To one who has followed even in a cursory fashion the recent literature on plant cytogenetics, they contain nothing new, and they bear no relation at all to the other contributions to the colloquium. Theodor Just, in a brief but comprehensive, up to date, and well organized summary of the origin and evolution of the flower, discusses recent concepts about the possible role of selection in molding this organ, as well as the present opinions of paleobotanists regarding the geological period and the ecological conditions under which the flowering plants arose. Dr. Just has been the most successful of the contributors in achieving a synthesis of information about both phylogeny and evolutionary dynamics.

The remaining contribution, that by Pierre Chouard on "Morphogenesis, Teratogenesis, and Evolution," represents a fresh and very interesting approach to the experimental study of morphology in relation to phylogeny. M. Chouard describes a series of experiments; first on the regeneration of buds and plants from pieces of leaves; second on teratological forms produced by the action of 2.4-D and other growth substances; and third on a most interesting series of modifications of leaf and flower structure produced by combinations of photoperiodic treatment combined with varying light intensities. Among them are examples of "neoteny," or the production of reproductive structures on tiny plants which bear leaves resembling cotyledons. The differences between some of these environmental modifications and the form of the same genotype grown under normal conditions are profound, and are strikingly parallel to some of the genetic changes which flowering plants have undergone during their evolution from one species or genus to another. Fuller understanding of these modifications should certainly give us a better insight into the mechanism of action of those genetic changes which have been important in phylogeny.

In his interpretations of these results M. Chouard becomes less convincing than in presenting the results themselves. He appears to be so much impressed by the parallelisms between environmental modification and those genetic changes which have been important in evolution that he conceives of the latter as having resulted from "directed mutations." As evidence in favor of the latter, he cites the "directed mutations" known to exist in the Bacteria, presumably the transformation phenomena in the Pneumococci. He apparently considers that such changes are of the same order as changes in the external form of a flowering plant, if one considers their physico-chemical basis, the only differences being caused by the greater complexity of development in the higher organisms. He therefore believes that a careful study of induced modifications, particularly neoteny, may lead to man's ability to produce directed mutations in higher organisms as well as in bacteria.

In this reviewer's opinion, M. Chouard is over optimistic. All of the evidence of modern genetics points toward the great length and complexity of the chain of reactions leading from the gene to the external character, and to the multiplicity of ways in which it may be broken or altered. For examples, one needs only to consider the great number of completely different, independently acting genes which contribute toward the formation of a normally shaped wing or a normally pigmented eye in *Drosophila,* or toward the formation of functioning chloroplasts in maize, barley, and probably every other higher plant. There is every reason to believe that the environmental modifications produced by M. Chouard and other experimenters are acting through entirely different channels from most or all of the mutations which have superficially similar end effects.

There is another reason for questioning the pertinence of M. Chouard's reference to induced changes in bacteria. These changes have been produced by only one type of agent; namely a "transforming substance" which resembles the chemical substances found in the nucleus, and which is derived from an organism similar to the end point of the change. To conceive of a similar type of directed mutation in higher plants, one would have to imagine the induction of mutations in the direction of, say, a prostrate form of a seacoast inhabiting type not through the action of any agent of the external environment present on the seacoast, but through the activity of nuclear material derived from a plant already adapted to this habitat. Such an influence can be imagined in nature only through the familiar processes of hybridization and selection.

The parallelism between certain environmental modifications and some of the genetic changes which have modified plant species in an adaptive direction can, in this reviewer's opinion, be explained on an entirely different basis from that proposed by M. Chouard. We might expect that in any well adapted organism the integration of gene action in development would be such that alterations in such ever present external factors as light and temperature would tend to produce harmonious alterations in the

phenotype, enabling it to grow and reproduce as well as possible under the altered conditions. Similarly, in the evolutionary adaptation of a race or species to a new environment, only those mutations would be selected which would enable the organism to react harmoniously to the new environment, thus producing a functioning, reproducing individual. Thus the manner of reaction of an individual to different environments which it meets during its lifetime as well as the response of an evolving race subjected to new environments during geologic time could both result from the action of natural selection. Mutations which produce disharmony under any of a wide range of environmental conditions tend to be rejected by selection, leaving in the population only those which produce harmony. Furthermore, one must consider the possibility or even the probability that the plant species in which M. Chouard has been able to produce neotenous and other modifications are a selected group, in which for some reason or other mutations enabling the genotype to react more or less harmoniously to a wide variety of apparently abnormal environments have had a particularly high selective value. Anyone who has grown many different kinds of plant species under a variety of environmental conditions knows that some have great individual plasticity, responding to different environments by producing phenotypes which differ greatly from each other; while other species are much more stereotyped, so that they will not grow at all except under a narrow range of environments, and so produce only a narrow range of different phenotypes. One can hardly doubt that these differences in the range of reaction of different genotypes are themselves genetically controlled; and our present knowledge about evolutionary dynamics in groups which have been studied experimentally indicates that such changes have originated via the selection of favorable mutations or gene combinations. The experiments of Dobzhansky and Wallace on the maintenance of homoeostasis by selection for coadapted genotypes in populations of *Drosophila* provide strong evidence in favor of this point of view.

Some of the data presented by M. Chouard himself definitely favor this point of view. While the modifications produced by altered photoperiods and light intensities resemble differences between natural species and genera, those produced by the action of 2.4-D, a chemical substance to which plants are not normally exposed, are mere teratological abnormalities, which in no way resemble differences between natural entities, but which do occasionally appear in nature, either as a result of some other abnormal environmental agent, or as occasional mutations, which are consistently rejected by natural selection. We can therefore conclude that natural selection has through the ages molded these species into collections of genotypes with adaptive reaction norms by selecting from the multitude of random genetic changes those which enable the genotype to produce a vegetatively and reproductively functioning phenotype under conditions relatively similar to those which it ordinarily finds in its range of natural habitats, but not under the action of external agents completely foreign to it.

Nevertheless, in spite of disagreements with his interpretations, the progressive student of plant evolution must congratulate M. Chouard for breaking away from idealistic tradition, and for recognizing that we can ultimately understand phylogeny only in terms of gene controlled alterations of the biochemical and biophysical processes of development, rather than postulating undefined "modifications" of putative "ancestral types" of adult organs.

The same point of view is developed much further in another far more significant volume by C. W. Wardlaw.[2] This author has declared that his purpose is (p. 2) "to consider what is involved in the study of phylogeny and morphogenesis, and to indicate, in broad outline, what has so far been achieved; to examine the relation of the two aspects; and to inquire how such conclusions as may emerge are likely to affect our views on the process of evolution."

This is surely a much needed task, and nobody is beter equipped to carry it out than Dr. Wardlaw, whose brilliant experiments on microdissection of the growing points of ferns have opened up a new avenue of approach to studying the relationship between the development of organs as seen from without and the physicochemical processes which are forming them from within. His failure to arrive at far reaching conclusions concerning the nature of this relationship is evidence not of his lack of qualification for such a study, but of the pitiful inadequacy of our knowledge in this field.

Dr. Wardlaw's book begins with a review of our present knowledge about the phylogeny of the ferns and their allies, derived from paleontology and comparative morphology. He then sets the stage for an analysis of the processes which have governed this phylogeny by describing the basic structure of the embryo and apical meristem in ferns.

His analysis of morphogenetic processes includes in turn a review of the evidence concerning them derived from genetics, biochemistry, physical factors, and mathematics. Each of these sections is well developed, and contains abundant references to the most recent literature, as well as to classical works such as those of Hofmeister, Sachs, and D'Arcy Thompson. Nevertheless, after reading these extensive reviews,

[2] Wardlaw, C. W. 1952. Phylogeny and morphogenesis. 536 pp. London, Macmillan Co.

one gains little sense of continuity or integration. In the three chapters dealing with genetics and cytology, for example, the reader is introduced to theories of the nature of the gene, to cytoplasmic inheritance, to the biochemistry and timing of gene action, to a series of extreme teratological mutations, to the structure of certain hybrids, polyploids, and chimaeras, to the genes which regulate allometric growth constants, and finally to theories concerning the role of gene action in differentiation, without his becoming aware of any connections between different topics. As a geneticist, this reviewer feels that far too little attention was paid to a phenomenon which in his opinion should become the main theme of any discussion aiming to establish a relation between gene action and morphogenesis; namely to the multiple end effects produced by many genes. If we accept the dictum, for which much evidence is now available, that each gene has but one primary function, then the best way of finding out how some primary biochemical process is acting to mold adult form would be to identify biochemically some genetically controlled alteration of a primary process, and to trace out as exactly as possible the way in which this chemical change is altering the adult form of the various organs produced by the primary meristem. The potentialities of such an approach seem to have been overlooked by Dr. Wardlaw.

After discussing the separate processes of morphogenesis, Dr. Wardlaw turns to experimental evidence on the nature of the shoot apex, emphasizing chiefly his own work on ferns, but reviewing also the work of Ball and others on flowering plants. He makes a convincing case for the totipotent nature of the apical meristem, and for the hypothesis that the phyllotactic arrangement of leaves results from the distribution of growth centres, fields, and gradients, which are initiated by the apical meristem, and which result from its morphogenetic and genetic constitution. The origin and development of leaf shape is treated only briefly, while the morphogenesis of the reproductive organs is not discussed at all. There is also no discusion of the possible morphogenetic basis of the general phylogenetic trends recognized by many morphologists, such as reduction of organs, fusion of parts, and changes in symmetry. In fact, this reviewer notes a general tendency on the part of Wardlaw to overlook or minimize the comparative approach to morphogenetic problems.

The final section of Dr. Wardlaw's book is a review of theories of morphogenesis, and an attempt to synthesize the available evidence. The strictly morphological theories are discarded as being inadequate even to serve as stimuli for further progressive research, and emphasis is placed upon physiological concepts, such as the gradient concept of Child, the field concept of Spemann, Gurwitsch, and Weiss, and the physicochemical concepts of protoplasmic organization advanced by Needham. These concepts were all originally worked out for animal development, but the microdissection experiments of Wardlaw and others on shoot apices have shown that they may be applied equally well to plants. In fact, the recognition of the fundamental similarities between the mechanisms of plant development and of animal development is one of the most important contributions of the book.

After reading these last two chapters, this reviewer is impressed again with our lack of knowledge of the subject. Any decision as to which of the various theories discussed comes nearest to the truth must definitely be left to the future. Meanwhile, the geneticist and morphologist interested in evolution can see in most of them stimulating ideas for future research. The more we abandon the search for idealistic concepts to express relationships between adult organs, and instead devote our attention to learning about the patterns of gene controlled chemical and physical processes which must be controlling development, the greater will become our fundamental knowledge and the wider will open our vistas for further research.

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A FIRST READER IN EVOLUTION [1]

R. G. JOHNSON

Here is an elementary textbook designed to bring the general reader and the beginning student face to face with the greatest general principle in biology. It is an honest book, aspiring only to equip its audience with some measure of understanding rather than to supply all of the intricate ramifications of its subject.

After issuing the reader a few necessary staples, the author establishes the fact of change by presenting examples from major areas of zoological research. From morphology, physiology and paleontology the evidence is marshalled in defense. No less than twelve chapters are devoted to the cause. A welcomed addition to

[1] Moody, P. A. 1953. Introduction to evolution. Harper & Brothers, New York: i–xii; 1–475, 152 figs.

the usual treatment is the lucid discussion of evolution as seen in chemical structure and metabolism. Following the fact of change, the mechanisms and processes involved are then considered. This section reaches its climax in a well presented chapter on the genetics of populations. The text is concluded with an open letter to students, being the authors personal conciliation of the concept of evolution and modern religious thought.

This is a well written first reader in evolution. The approach is not new, though some of the examples listed are and one might well question whether or not the good writing is pulled down by a rather pedantic organization of materials. The animal gets chopped up into its embryology, its chemistry etc. as the artificial compartments of biological endeavor are superimposed upon the study of evolution. The reader is confronted with a myriad of boxed up facts which are not related until the last moment and he might well be encouraged by a little more substantial plot. This reviewer found the author's attempt at meeting the philosophical problems posed the young student by evolution, a frank and realistic approach to a real problem too often avoided by academic pussyfooting. As a text it is difficult to see just where it might fit in its entirity in the curriculum of the beginning student, but it is a good first reader, worthy of any teacher's consideration.

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